NERL/MCEARD Publications

Technical Information Manager: Angela Moore (513) 569-7341

Jan 1, 2003 - Dec 31, 2003 ***Presented Published***

ABSTRCT/ORAL

Hedrick, E.J., Slingsby, R., Munch, D.J., and Hautman, D.P. Low-level determination of perchlorate in drinking water using ion chromatography mass spectrometry. Presented at: 51st ASMS Conference, Montreal, Canada, June 8-12, 2003. 6/8/2003

Contact: Elizabeth J. Hedrick

Abstract: Perchlorate is a drinking water contaminant originating from the dissolution of the salts of ammonium, potassium, magnesium, or sodium in water. It is used primarily as an oxidant in solid propellant for rockets, missiles, pyrotechnics, as a component in air bag inflators, and in highway safety flares. Based on EPA Information Request Responses, there are 44 states that have reported perchlorate manufacturers or users. From accidental releases and disposal, perchlorate has become a contaminant in surface and ground waters where it is highly mobile and, due to its chemical stability, persists for decades. The primary human health effect is inhibition of iodide uptake by the thyroid gland. By disrupting thyroid hormone production, perchlorate interferes with metabolism and can affect brain development in fetuses and children, leading to mental impairment. The perchlorate anion (ClO4 -) has been found in numerous drinking water supplies at concentrations that recent studies indicate may adversely affect human health. There is an urgent need to be able to confirm and quantify perchlorate at lower concentrations than the currently approved U.S. EPA method allows. In this work, sub-ppb quantitation of perchlorate in drinking waters using ion chromatography with conductivity suppression, electrospray ionization mass spectrometry (IC-ESI-MS) was demonstrated. The primary mass of interest is 99 based on the 75.77% relative abundance of the chlorine-35 isotope. Mass 101, derived from the 24.23% abundance of chlorine-37, is a secondary mass that was also utilized for quantitation and confirmation. Low-level linear calibrations from 0.01 - 1.0 ppb, yielded R2 >0.999. Method detection limits (MDLs) in deionized and high ionic waters (up to 1000 ppm common anions sulfate, chloride and carbonate) were from 0.03 - 0.11 ppb with no significant difference, at alpha=0.01, between mass 99 and mass 101 MDLs. Precision of replicate injections at 1 ppb, yielded <5% relative standard deviation on mass 99 and <5% on mass 101 on a daily basis. Accuracy as determined by analysis of a certified reference material was +5% of the certified value in deionized water. Ruggedness, as determined by the reproducibility of area counts of 1.0 ppb check standards analyzed periodically over a day of continuous analysis of high ionic matrices, revealed some deterioration of signal intensity (~15% drop). The major cause of a loss of sensitivity was fouling of the MS sampling cone. Rapid fouling occurred during the first five minutes of analysis due to the elution of cations and common anions such as chloride, sulfate and nitrate. It is also during this time frame that the eluate pH dips to pH 1 due to H+ exchange with monovalent cations that occurs in the electrolytic conductivity suppressor. This highly acidic eluate can damage the stainless steel capillary and sampling cone. To slow column fouling, mass spectrometer manufacturers often recommend diverting the LC flow to the MS until just prior to the elution of the analyte of interest (matrix cutting). Matrix cutting proved to be beneficial for extending the period between cone cleanings and in maintaining signal intensity over the course of a day. A chromatographic problem observed was tailing of large concentrations of the anions sulfate, chloride and nitrate into the elution time of ClO4-. Due to a minor isotope of sulfur, HSO4 - (mass 99) interferes spectrally with ClO4-. The best way to eliminate the problem is to remove the sulfate prior to sample analysis using precipitation with barium. Recoveries in three different tap waters ranged from 99 -102% based on the average recoveries from quantitation at masses 83, 99 and 101. In-source collisionally activated dissociation of ClO4- to yield ClO3- was done by increasing the cone voltage and was used as additional confirmation of ClO4 - .Contaminated ground waters with suspect concentrations of ClO4- (based upon previous analyses using IC with conductivity detection) were analyzed by the IC-MS method. The IC-MS method revealed that the suspect contaminants were not perchlorate. One site yielded low spike recovery which is believed to be due to ionization suppression from the combined effect of high sulfate and another contaminant with the same retention time as ClO4 -Future work will focus on finding a suitable internal standard and in exploring whether other mobile phases and separator columns can achieve the same performance. Isotope dilution calibration using an enriched ClO4- standard (enriched on 18-O) will be tested in the near future.Overall, IC-suppressed conductivity-ESI-MS proved to be sensitive and specific for ClO4- in drinking and ground waters at sub-ppb concentrations.

Hedrick, E.J. Methods development to improve low-level perchlorate detection in drinking water by conductivity and mass spectrometry - issues and impact. Presented at: Severn Trent 4th annual Analytical Program Compliance, Louisville, KY, June 17-18, 2003. 6/17/2003

Contact: Elizabeth J. Hedrick

Abstract: The goal of this research is to develop a USEPA method for the determination of sub-ppb concentrations of the perchlorate anion in ground and surface drinking waters. To date, ion chromatography using a KOH mobile phase, electrolytic conductivity suppression and electrospray ionization mass spectrometric detection has been explored. Perchlorate is a drinking water contaminant originating from the dissolution of the salts of ammonium, potassium, magnesium or sodium in water. It is used primarily as an oxidant in solid propellant for rockets, missiles, pyrotechnics, as a component in air bag inflators, and in highway safety flares. Based on EPA Information Request Responses and occurrence monitoring, there are 95 confrmed perchlorate releases in 25 states and 230 users or manufacturers in 40 states. From accidental releases and disposal, perchlorate has become a contaminant in surface and ground waters where it is highly mobile and, due to its chemical stability, persists for decades. The primary human health effect is inhibition of iodide uptake by the thyroid gland. By disrupting the thyroid hormone production, perchlorate interferes with metabolism and can affect brain development in fetuses and children, leading to mental impairment. The perchlorate anion has been found in numerous drinking water supplies across the United States. There is an urgent need to be able to confirm and quantify perchlorate at lower concentrations than the currently approved USEPA method allows which use ion chromatography with suppressed conductivity detection. In this work, sub-ppb quantitation of perchlorate in drinking waters and contaminated ground waters using ion chromatography with electrolytic conductivity suppression, electrospray ionization mass spectrometry (IC-ESI-MS) is demonstrated.

Morgan, J.N., Hieber, T., Kauffman, P., and Brisbin, J. Determination of pesticides in comosite diets using large volume pressurized fluid extraction with in-line sample preparation. Presented at: 40th Annual AFDOSS Florida Pesticide Residue Workshop, St. Petersburg, FL, July 20-23, 2003. 7/20/2003

Contact: Jeffrey N. Morgan

Abstract: USEPA's National Exposure Research Laboratory conducts research to measure the exposure of individuals to chemical pollutants through the diet, as well as other media. In support of this research, methods are being evaluated for determination of various classes of pesticides in composite diet samples. In previous work, pressurized fluid extraction (PFE) followed by diatomaceous earth and C18 reversed phase column chromatography was used in the determination of organophosphate pesticides in composite diet samples. PFE followed by diatomaceous earth and alumina column chromatography was used for a diverse mix of organochlorine and other pesticides. The current study evaluated an automated system for performing the extraction and cleanup in one step in a 100 mL PFE cell. Various combinations of sample amounts, extraction solvents and adsorbents were tested. Organophosphate pesticides were quantitated by gas chromatography with pulsed flame photometric detection. Organochlorine pesticides were quantified by gas chromatography/mass spectrometry in the selected ion monitoring mode. Results of this study demonstrated this automated procedure, using acetonitrile, a super absorbent polymer and C18, was comparable to the more laborious PFE/column chromatography methods used previously. Acetonitrile replaced a mixed solvent system of acetone/methylene chloride used in previous studies. Recoveries for most pesticides fell within the target range of 60 to 140%. Results obtained for organochlorine pesticides are preliminary and additional work is needed to optimize this system for those analytes.

Miller, T.A., and Schaefer, F.W. Characterization of a mouse model of iimmunosuppression using a single does of methylprednisolone acetate. Presented at: Society of Comparative Endocrinology, Asheville, NC, June 1-4, 2003. 6/1/2003

Contact: Frank W. Schaefer

Abstract: Immunosuppression is a widely used but rarely defined term. To the transplant specialist it is the state at which the transplanted organ is not rejected. For the HIV patient it is the point when AIDS begins. In research, immunosuppression is achieved through the use of glucocorticoids, usualy for prolong period of time. In this model a single dose of methylprednisolone acetate (Depomedrol) was used to produce immunosuppression. The circulating CD3, CD4 and CD8 lymphocyte levels were analyzed to characterize the immunologic changes induced. A drop of greater than 80% was needed to re-establish a Cryptosporidium muris infection in mice who had fully recovered from an initial infection. When compared, the ID50 for mice who were immunosuppressed was three times higher than the ID50 for normal mice, showing immunosuppressed mice were less susceptible to C. muris invasion.

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Schaefer, III, F.W. The establishment of laboratory guidelines for analysis of bioterrorism samples. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Frank W. Schaefer

Abstract:

Schaefer, III, F.W. Approaches to inactivation and federal regulations. Presented at: Workshop on Transport and Disposal of Wastes from Facilities Contaminated with Chemical and Biological Agents, Cincinnati, OH, May 28-30, 2003. 5/28/2003

Contact: Frank W. Schaefer

Abstract:

Miller, T.A., and Schaefer, F.W. Changes in mouse circulating t-lymphocyte numbers during a normal cryptosporidium muris infection and after a single injection of methylprednisolone acetate. Presented at: Experimental Biology Meeting, San Diego, CA, April 11-15, 2003. 4/11/2003

Contact: Frank W. Schaefer

Abstract: Cryptosporidium species are of public health significance in both developing nations and the industrialized nations of the world. Persons with immature or compromised immune systems are said to have increased risk or mortality once infected. The objective of this study was to chart circulating lymphocyte numbers before, during and after a natural C. muris infection and to analyze the effects of a single dose of methylprednisolone acetate (MPA) on oocyst shedding and T cell numbers. Thirty-two day old female CF-1 mice were exposed and monitored for oocyst shedding. CD3, CD4, and CD8 numbers were obtained using flow cytometry from sacrificed mice over the course of the infection. In a subset of mice MPA was injected on day 15 post exposure and both T-lymphocytes and oocyst shedding were monitored. Results showed a significant increase in the duration and numbers of oocyst shed after MPA. T-lymphocytes radically decreased by greater than 90% after MPA.

Schaefer, III, F.W. The establishment of laboratory guidelines for analysis of bioterrorism samples. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Frank W. Schaefer

Abstract: After the attack on the World Trade Center on September 11, 2002, and the subsequent deaths associated with Bacillus anthracis spore contaminated mail, a worldwide need was apparent for increased laboratory capacity to safely analyze bioterrorism samples. The U.S. Department of Health and Human Services has furnished guidelines for microbiological and biomedical laboratory safety. These guidelines encompass laboratory practices and techniques, facility design, safety equipment, monitoring the analyst's health, vermin and insect control, as well as government control of select agents and specialized reagents. Before work is initiated, the laboratory must have protocols which cover all standard operating procedures, quality assurance, chain of custody, and a detailed biosafety plan. Special emphasis is placed on the approach used in the event of either an accidental spill or accidental exposure. Extensive training in all aspects of the protocols is required for each analyst. Great care also is used to document both how and who processed the samples in case they have forensic significance.

Design of the laboratory facility centers around containment and segregation of the sample analyses, so as few people as possible are involved. Persons who are immunocompromised should not under any circumstances be permitted access to such a hazardous facility. Key card access through an airlock allowing only authorized personnel into the laboratory helps ensure such a policy. All laboratory benches must have impervious surfaces. The walls and floors also should be sealed, so liquids cannot penetrate them. The laboratory equipment and benches should be set up in a fashion to allow routine cleaning and disinfection. There must be a sink in each laboratory to facilitate hand washing. Interlocking double door autoclaves, a specialized negative pressure ventilation system, and waste stream treatment must all be part of the design. The required safety equipment depends upon the type of analysis being performed. Generally gloves, laboratory coat or gown, shoe covers or boots, and eye protection are required. If the protocol entails analysis of a sample that is prone to the production of an aerosol, then a respirator is mandated. Most if not all procedures should be conducted in a biosafety cabinet. When centrifugations are necessary, they should be done with capped containers that in turn are placed in safety centrifuge cups. These cups are designed to prevent aerosols from being released during centrifugation. A biohazard warning sign naming the organism and incorporating the international biohazard symbol must be posted on all laboratory doors.

Shoemaker, J.A., and Glassmeyer, S. The application of mass spectrometry to the study of microorganisms. Presented at: U.S. EPA's Research on Micoorganisms in Drinking Water Workshop, Cincinnati, OH, August 5-7, 2003. 8/5/2003

Contact: Jody A. Shoemaker

Abstract: The purpose of this research project is to use state-of-the-art mass spectrometric techniques, such as electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS), to provide "protein mass fingerprinting" and protein sequencing information for microorgansims listed on the 1998 Contaminant Candidate List (CCL) that cause waterborne disease. The responsibility of characterizing and investigating microorganisms has traditionally fallen to microbiologists, but recent advances in mass spectrometry have allowed analytical chemists to also enter the realm of microorganisms.Protein mass fingerprinting libraries will be developed and evaluated to determine whether MS techniques can identify protein fingerprints related to the infectivity/viability of selected microorganisms and whether they can differentiate between species and strains of selected microorganisms. Sequence information for proteins which are found to be specific or unique to species/strain and infectivity/viability can also be obtained with these MS techniques. This global proteomic project has a number of subtasks for which preliminary results have been obtained on microorganisms such as coxsackievirus, Cryptosporidium parvum, and enterococci. Through the use of mass spectrometry, a potential viral biomarker of coxsackievirus has been identified which may indicate whether the virus is infectious. A unique mass spectral peak was observed in an infectious coxsackievirus, but was not observed in a non-infectious coxsackievirus. This unique peak may be responsible for viral infectivity, thus, be a potential biomarker. In addition to viruses, initial experiments were performed to determine the ability of MALDI to analyze C. parvum both in an intact form, as well as oocysts that have been rendered nonviable. MALDI analysis was performed on several different harvests of the intact oocysts, as well as the separated cell walls and sporozoites that make up the oocysts. The analysis of the oocysts walls was inconclusive due to lack of discernable mass spectral peaks, but MALDI analysis of the sporozoites yielded reproducible mass spectra. Whole enterococci cell protein profiles were evaluated using MALDI as a tool to identify seven different enterococci species. Many mass spectral peaks were shared among the different enterococci species, however, each species showed unique peaks, primarily in the 6,000 to 7,000 m/z region. When environmental isolates were tested, the signature peaks were observed in many of the different isolates, suggesting that these peaks could be used for species identification. Sequence analysis of the environmental isolates by 16S rDNA confirmed the identity of the strains tested, and matched the MALDI identity prediction in 75 % of the samples. The results from this study indicate that the analysis of whole enterococci cells by MALDI generate unique protein profiles which can be used for the rapid identification of fecal enterococci environmental isolates. Although mass spectrometry currently is not sensitive enough to detect single cells in drinking water, the basic proteomic information obtained with these mass spectrometric techniques can be used to develop more sensitive and precise microbiological techniques that focus on these unique proteins in drinking water samples. These conventional microbiological methods can then be used to gather the occurrence data that will be used to create better EPA regulations for protecting humans from microbiological contaminants in U.S. drinking water supplies.

Shoemaker, J.A. The application of mass spectrometry to protein analysis. Presented at: NHEERL Presenation on Proteomics, Research Triangle Park, NC, July 22, 2003. 7/22/2003

Contact: Jody A. Shoemaker

Abstract: The purpose of this presentation is to give our NHEERL collaborators a brief introduction to the use of mass spectrometric (MS) techniques in the analysis of proteins. The basic principles of electrospray ionization and matrix-assisted laser desorption ionization will be discussed, as well as the use of tandem mass spectormetry for de novo sequencing of novel proteins. This information will aid our collaborators in understanding the proteomic data we will be providing them on allergenic fungal proteins extracted from Metarhizium Anisopliae.

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Presented Published

Donohue, M.J., Shoemaker, J.A., Selgrade, MJ, Ward, M.D.W., and Copeland, LB. Proteomic analysis of allergens from metarhizium anisopliae. Presented at: 51st ASMS Conference, Montreal, Canada, June 8-12, 2003. 6/8/2003

Contact: Jody A. Shoemaker

Abstract: IntroductionThe goal of this project is the identification and characterization of allergens from the fungus Metarhizium anisopliae, using mass spectrometry (MS). The US EPA, under the "Children at Risk" program, is currently addressing the problem of indoor fungal bioaerosol contamination. One of the research objectives is to develop a basic understanding of IgE inducing proteins from fungi, using advanced proteomics. The fungus M. anisopliae has been used as a bio-pesticide for insect control since the 1800's. Recent studies have shown that exposure to this microorganism can cause an immediate hypersensitivity or Type I allergic responses in mice1. Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization tandem mass spectrometry (ESI-MS/MS) were used to analyze the fungal peptides and proteins isolated by 2-D gel electrophoresis and immuno-blot analysis. The MS data on molecular weight, peptide profiles, and amino acid sequence were used to mine databases for potential sequence or domain homology to known allergens. This information will provide better characterization of the M. anisopliae proteins that induce IgE responses, eventually paving the way to a better understanding as well as the prevention and/or treatment of protein allergies. Method Sample PreparationThe fungal extract preparation was preformed following a modified method described by Stankus and O'Neil2. Metarhizium anisopliae was grown under three different preparations. The proteins were extracted, and assayed for total protein concentration. Equal protein concentrations of each growth condition were combined to create the M. anisopliae crude antigen (MACA) preparation.2D gel Electrophoresis and Immuno-blot AnalysisTotal protein extracts of M. anisopliae were separated using 2-D gel electrophoresis. Allergenic proteins were identified by immuno-blot analysis, using hyperimmune mouse serum against M. anisopliae extract as the primary antibody and anti-mouse IgE as the secondary antibody. Protein spots identified as inducing IgE were excised and in-gel digested. The peptides were extracted and analyzed by MALDI-TOF-MS and ESI-MS/MS. Peptide sequences were analyzed using the MASCOT data mining algorithm.Mass SpectrometryAll MALDI analyses were acquired on a Bruker Biflex III. Samples were diluted 1:1 in matrix (a -HCCA, 10mg/ml; ACN 50% (v/v); TFA 0.1% (v/v). One microliter of this solution was spotted onto the MALDI target. All nanospray ESI/MS/MS data were acquired using an Applied Biosystem QSTAR XL equipped with a Proxeon nano-ESI source. Low-energy collision induced dissociation (CID) was preformed using nitrogen as the collision gas.Results and DiscussionEight proteins were identified as allergenic proteins, based on IgE reactivity in the immuno-blot assay. Five of the eight proteins had apparent molecular weights greater than 75 kDa. All the eight proteins have acidic pIs in the range between 4.8 and 5.5. At this time, all eight spots have been excised from the Coomassie stained gel. Protein fingerprints have been obtained for Spots 1, 2, and 5. The mass values have been used to mine the non-redundant databases to identify these allergenic proteins. Based on database mining, the proteins have been determined to be novel and are currently being sequenced by ESI-MS/MS. ConclusionIn this study, we have clearly demonstrated that exposures to M. anisopliae can induce an allergenic response by the identification of eight proteins that are reactive to IgE antibodies. Three of the identified proteins appear to be novel due to the lack of significant matches when mining the non-redundant databases. Efforts are ongoing to verify that the proteins are novel by amino acid sequencing the observed peptides. Reference1. Ward, M.D.; Sailstad, D.M.; Selgrade MJ.K. Toxicol. Sci. 1998, 4,195-203.2. Stankus, R.P.; O'Neil, C.E. J. Allergy Clin. Immunol. 1988, 81, 563-570.

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Shoemaker, J.A., Talley, J.M., Dahling, D.R., and Fout, G.S. The discovery of biomarkers of viral infectivity by mass spectrometry. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Jody A. Shoemaker

Abstract: Over the past three decades, the CDC and the U.S. EPA have collected and reported data relating to occurrences and causes of waterborne-disease outbreaks in the United States. Thirty nine outbreaks associated with drinking water were reported during 1999-2000. According to CDC's 1999-2000 water surveillance report, of the 20 outbreaks with known infectious causes, four were caused by viruses, which indicates that viral contamination of drinking water is a problem. A number of these viruses (e.g. coxsackie) are now listed on the U. S. EPA 1998 Contaminant Candidate List as needing additional research (i.e., analytical methods, occurrence, treatment and health effects). Coxsackievirus causes flu-like symptoms including diarrhea and fever and can cause complications such as diabetes mellitus and myocarditis. Thus, NERL is striving to develop methods for viruses listed on the CCL. This research project is a collaborative effort between NERL microbiologists and chemists to use analytical chemistry to differentiate between virus strains and to identify potential biomarkers of viral infectivity. Typical microbiological methods require months to determine the virus strain. Through the use of mass spectrometry, we have identified potential viral biomarkers which can be used to differentiate rapidly between the strains of coxsackievirus and can potentially indicate whether the virus is infectious. A unique peak was observed in an infectious coxsackievirus, but was not observed in a non-infectious coxsackievirus. This unique peak may be responsible for viral infectivity, thus, be a potential biomarker of infectivity. This type of information, gathered through the use of mass spectrometric techniques is cutting edge, ground breaking basic research, and can have a significant impact on the field of virology and play a role in the future development of drinking water methods to detect viruses and their infectivity.

Shoemaker, J.A., Talley, J.M., Dahling, D.R., and Fout, G.S. The discovery of biomarkers of viral infectivity by mass spectrometry. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Jody A. Shoemaker

Abstract:

Martinson, J., Lewis, D., Brenner, K., Wymer, L., and Brown, S. Information management and related quality assurance for a large scale, multi-site research project. Presented at: The U.S. EPA 20th Annual Conference on Managing Environmental Quality Systems: "Quality Management Solutions for Today's Environmental Challenges, New Orleans, LA, April 14-17, 2003. 4/14/2003

Contact: John W. Martinson

Abstract: During the summer of 2000, as part of a U.S. Environmental Protection Agency study designed to improve microbial water quality monitoring protocols at public beaches, over 11,000 water samples were collected at five selected beaches across the country. At each beach, samples were collected at least twice daily according to one or four sampling schemes. Samples were delivered to near-by laboratories and analyzed the same day for either E. coli or Enterococcus by established microbiological methods. Locational data, data describing ambient conditions at the beaches, and other supporting environmental data were also collected. The staff at each of the five study sites reported their data daily to a central location.The collective effort generated close to a half-million data points during the months of July and August. Managing the steady and complex flow of informatiton and assuring the quality required a well planned, well-organized, and innovative approach. This approach included:. standardized paper and electronic data entry forms,. centrally-provided waterproof sample labels with pre-printed sample identifiers,. independent, double entry of data with automated comparison,. adherence to basic relational database rules, and careful attention to good data structure design, and data management.This presentation will report in detail the data management approach, explain the reasoning behind the approach, and explain how it was integrated with the QA Project Plan. The speaker will describe procedures used to assess data quality in both the electornic forms completed at the sampling locations and the central database. The speaker will analyze the costs and benefits associated with the chosen approach, and will evaluate its success. Future plans (long-term archival, public availability, etc.) for the data will be described.

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Budde, W.L., and Maizels, M. Investigatoin of cyanobacteria toxins in water. Presented at: Annual Conference of the American Society for Mass Spectrometry, Montreal, CA, June 8-13, 2003. 6/8/2003

Contact: William L. Budde

Abstract: Introduction:Approximately 80 alkaloid and cyclic peptide toxins produced by various freshwater and marine cyanobacteria (blue-green algae) have been identified and their structures determined. The U. S. Environmental Protection Agency has identified two neurotoxin alkaloids and four cyclic peptide toxins that have the potential for serious contamination of sources of drinking water for humans and animals. The purpose of this research is to develop a convenient, rapid, but definitive analytical method for these toxins in water.Methods and Instrumentation:Four of the six toxins of interest, and three of lessor interest, were available for this work. Anatoxin-a, microcystins RR, LR, and YR, and nodularin were added to laboratory reagent water and to samples of natural waters at environmentally significant concentrations. The analytes were separated from the water by liquid-solid extraction using C-18 silica impregnated filter disks, eluted from the disks with methanol, and the eluate was analyzed using microbore (1 mm ID) LC combined with electrospray and time-of-flight mass spectrometry.Preliminary Data:The analytes anatoxin-a, microcystins RR, LR, and YR, and the cyclic peptide nodularin in methanol are separated in about 25 minutes. With about 50 ng of each injected, the LC peaks in the TIC have very good S/N. Microcystin RR gives mainly the (M+2H]2+ ion and the other analyes give the corresponding singly charged ions. In source CID gives a characteristic m/z 135 ion for the microcystins and nodularin, but anatoxin-a does not have the necessary structural feature to give this ion. Mean recoveries from laboratory water for the microcystins and nodularin were in the 85-98% range with RSDs in the 5- 17% range. The mean recovery of anatoxin-a was just 68% (RSD 11%). A suitable internal standard has not been identified and external standardization may account for some of the recoveries and analytical presicion.

Parks, A.N., Gallagher, P.A., Schwegel, C.A., Ackerman, A.H., and Creed, J.T. An investigation of arsenic mobility from iron oxide solids produced during drinking water treatment. Presented at: 2003 Pittsburgh Conference, Orlando, FL, March 9-14, 2003. 3/9/2003

Contact: John T. Creed

Abstract: The Arsenic Rule under the Safe Drinking Water Act will require certain drinking water suppliers to add to or modify their existing treatment in order to comply with the regulations. One of the treatment options is iron co-precipitation. This treatment is attractive because arsenic and iron are geologically correlated so that well waters containing arsenic have a propensity to contain iron. Iron can be precipitated from water via aeration and in the process the iron co-precipitates some of the arsenic leading to arsenic removal. The aeration can take place during the treatment process or occur as the water is pumped from the supply through the distribution system. The presence of these iron oxide solids can alter the distribution of the naturally occurring As(III) and As(V) in the water. The stability of the resulting iron oxide/arsenic complex ("FeOOHAs") is not well characterized. This research will attempt to estimate the distribution of As(III) and As(V) on these iron oxide solids and estimate the stability of these complexes produced by changing water quality parameter. The arsenic distribution on the solids will be estimated by extraction with acetic acid followed by IC-ICP-MS separation and detection. The stability will be estimated by conducting total metal measurements on solids in contact with waters prior to and after changes in pH, nitrate and chlorine concentrations

Creed, J.T., Gallagher, P.A., and Schwegel, C.A. Are all arsenic exposures toxic? Supporting regional risk assessments through improved arsenic speciation methodology. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: John T. Creed

Abstract:

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Parks, A.N., Gallagher, P.A., Schwegel, C.A., Ackerman, A.H., and Creed, J.T. The liberation of arsenosugars from matrix components in difficult to extract seafood samples utilizing TMAOH/acetic acid sequentially in a two-stage extraction process. Presented at: 2003 European Winter Conference on Plasma Spectrochemistry, Garmisch-Partenkirchen, Germany, January 12-17, 2003. 1/12/2003

Contact: John T. Creed

Abstract: Sample extraction is one of the most important steps in arsenic speciation analysis of solid dietary samples. One of the problem areas in this analysis is the partial extraction of arsenicals from seafood samples. The partial extraction allows the toxicity of the extracted arsenicals to be determined but the unextracted fraction's toxicity can not be determined. This inability to determine the toxicity of the unextracted arsenicals creates a relatively large source of uncertainty in estimating the risk from seafood exposures. Seafood exposures can produce a relatively large source of uncertainty because the arsenic concentrations in seafoods can be orders of magnitude higher than those associated with other food groups and therefore, a small error in quantitating toxicity (unextracted arsenicals imply unknown toxicity) in seafoods produces larger uncertainties in the overall exposure assessment. An additional problem can be generated by non-quantitative extractions in which the extraction solvent preferentially removes the non-toxic species while leaving the toxic species unextracted. The interpretation of these data would be that the sample contains only non-toxic arsenicals when in reality the extraction solvent has selectively removed only the non-toxic species. In this case the solvent selectivity produces an analytical negative bias which underestimates the concentration of the toxic arsenicals. Thus, a quantitative extraction is needed to minimize the unextractable arsenicals (which should provide the maximum species specific information) and eliminate the question about analytical bias induced by solvent selectivity (since all the arsenic is accounted for). This presentation will focus on the use of tetramethylammonium hydroxide (TMAOH) as an extraction solvent for difficult-to-extract seafoods. The extraction utilizes a 0.83% TMAOH extraction solvent which is heated in a convection oven at 60oC for 3hrs. The sample is then neutralized with acetic acid to remove proteins and returned to the oven for 21hrs at 80oC. Data will be presented which indicates that the neutralization step is essential because arsenosugars are converted during this process from an unchromatographable species to a chromatographable species. Chromatographic and total arsenic data will be presented to document this conversion. Finally, chromatographic data will be summarized utilizing the two-stage (base followed by acid) extraction procedure.

Gallagher, P.A., Evans, O.M., Parks, A.N., Schwegel, C.A., Ackerman, A.H., Creed, J.T., and Wilbur, S. Speciation of non-pesticidal organotin compounds using gas chromatography with inductively coupled plasma-mass spectrometry. Presented at: 2003 European Winter Conference on Plasma Spectrochemistry, Garmisch-Partenkirchen, Germany, January 12-17, 2003. 1/12/2003

Contact: John T. Creed

Abstract: Organotins can be classified into a broad class of compounds referred to as endocrine disruptors and are presently being used in many applications such as UV stabilizers in polyvinyl chloride (PVC) pipes, antifoulants in marine paints, and as fungicides to name a few. The organotins used in PVC pipes are a growing concern given the increased use of PVC pipe in drinking water distribution systems. Preliminary studies have indicated that PVC pipe initially releases a large percentage of its organotins immediately after being placed into service and the concentration drops off with continued use. These data raise two issues: initial exposure severity and long-term low dose exposure effects of organotins. The first issue can be addressed rather easily using existing analytical methodologies. However, addressing the second issue requires the measurement of organotins at sub-ppb to fractional ppt, which is considerably more challenging. One of the most promising analytical techniques for the detection of organotins at extremely low concentrations involves gas chromatographic (GC) separation and inductively coupled plasma mass spectrometry (ICP-MS).This presentation will discuss the use of an Agilent GC-ICP-MS system for the detection of dimethyltin, monomethyltin, monobutyltin and dibutyltin. Preliminary performance data including detection limits and reproducibility at 5-10 times the method detection will be reported. This presentation will also provide isotope ratio performance data at low level concentrations utilizing the GC interface. The research findings will be presented in the context of the analytical requirements for PVC pipe leaching studies. Finally, data will be presented on the use of a liquid-liquid extraction as a means of pre-concentration prior to GC-ICP-MS detection.

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Ackerman, A.H., Gallagher, P.A., Parks, A.N., Schwegel, C.A., Creed, J.T., Heitkemper, D.T., and Vela, N. A mass balance approach to determine arsenic absorption rates from contaminated water by rice during the food preparation process. Presented at: 2003 European Winter Conference on Plasma Spectrochemistry, Garmisch-Partenkirchen, Germany, January 12-17, 2003. 1/12/2003

Contact: John T. Creed

Abstract: Rice represents a unique set of arsenic exposure assessment challenges in that it does contain a relatively high concentration of arsenic and it does absorb about 100% of its dry weight during food preparation. Arsenic exposure from consumption of rice can conceptually be divided into arsenic native to the rice [As(III), DMA, MMA and As(V)] and arsenic absorbed from the water [As(III) and As(V)] during food preparation. The arsenic exposure associated with the rice is a relative constant while the arsenic absorbed from the water may depend on the concentration of arsenic in the water used in food preparation and the way the rice is cooked (boiled or steamed). The actual exposure becomes difficult to calculate without knowing the native rice arsenic concentration, the arsenic concentration in the water and the percentage of arsenic absorbed during the food preparation process. The ability to estimate the native arsenic concentration in rice using a nearly quantitative extraction process has been reported [1] and the concentration of arsenic in the water is analytically fairly simple to determine. The unknown quantity is the absorption rates of the arsenic from the water to the rice. Given that rice is a common staple and that the water used for food preparation could represent a large percentage of the exposure dose, it is important to estimate the absorption rates in order to better characterize rice as an arsenic exposure source. This presentation will use an arsenic mass balance approach throughout the food preparation process in order to estimate arsenic absorption rates from water. Native arsenic concentrations will be determined for the rice, water and cooked rice. These data will be utilized along with percent moisture increase for the cooked rice to calculate the arsenic absorption rates. Total arsenic concentrations and speciation data will be acquired for individual components (rice, water, cooked rice) and the quality of the mass balance for boiled vs steamed rice will be discussed. All results will be based on replicate IC-ICP-MS analysis and the absorption rates will be estimated.

Creed, J.T. Are all arsenic exposures toxic? Supporting regional risk assessments through improved arsenic speciation methodology. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: John T. Creed

Abstract: Arsenic exposure assessments require the evaluation of the relative contribution of both media (water, food, etc.) and routes of exposure (ingestion, inhalation, dermal). For arsenic, the important media are predominately water and food and therefore, the route of concern for exposure is ingestion. In addition, the toxicity of an exposure is strongly dependent on the chemical form of the arsenic ingested. Water contains predominately toxic arsenic while foods contain a mixture of both toxic and non-toxic arsenicals. Thus, an accurate risk assessment must assess the exposure from water and food and must differentiate (or speciate) the toxic and non-toxic arsenicals present in foods. Furthermore, speciation of arsenicals in foods will aid in: 1) formulating an accurate relative source contribution (water vs. food), 2) conducting exposure studies to determine dose vs. response, and 3) help identify sub-populations which are highly exposed. These types of information become part of the scientific foundation used in formulating drinking water regulations Seafood has been identified as the major dietary contributor to arsenic exposure by the US FDA. US EPA's Region 10 (Alaska, Washington, Oregon, Idaho) seafood consumption rates are often well above average national values. For example, Native American (Washington and Oregon) and Alaska Native studies have indicated average seafood consumption rates up to ten times greater than the US EPA average estimate of 6.5 g/day. Thus, improved analytical methodology is needed to determine whether these sub populations are being exposed to toxic or non-toxic arsenic species. As a result, NERL has developed an analytical procedure for the speciation of arsenic in seafoods and transferred it to the Region 10 laboratory. This procedure is being used to generate a preliminary speciation based database for arsenic in seafoods and should aid regulators in estimating the relative source contribution of food vs. water. Ultimately, this will aid the Region in assessing the impact of their elevated seafood consumption rates.

Munch, J.W. Developing analytical methods for gathering nationwide occurrence data for chemicals on the Drinking Water Contaminant Candidate List (CCL). Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Jean W. Munch

Abstract: Amendments to the Safe Drinking Water Act (SDWA) require the United States Environmental Protection Agency (USEPA) to publish a list of contaminants that are known or anticipated to occur in public water systems, and which may require regulation under the SDWA. In response to this requirement, and after extensive consultation with the scientific community, the USEPA's Office of Ground Water and Drinking Water (OGWDW) published a Drinking Water Contaminant Candidate List (CCL) of 50 chemicals and 10 microorganisms in March 1998. A critical piece of information that OGWDW must have for making a regulatory determination on these contaminants is the frequency and magnitude of their occurrence in public drinking water systems across the country. This information will be collected by OGWDW through a nationwide chemical monitoring program at drinking water utilities. Because of the importance of the regulatory determination process, it is essential that the test methods used to gather this occurrence data be both sensitive and specific. NERL collaborated with OGWDW to determine which of the 50 chemicals under consideration for regulatory action needed new, improved analytical methods prior to collection of occurrence data. From the list of chemicals for which method development was required, NERL has completed the development of two analytical methods that together will test for five chemicals of concern, and meet the data quality objectives of OGWDW. Method 528 is currently being used in a nationwide monitoring study of public drinking water supplies to obtain data on four substituted phenols listed on the CCL, and on an additional eight substituted phenols that are priority pollutants. Phenols are of concern as drinking water contaminants because of their widespread use as intermediates in the manufacture of pesticides, bactericides, and other industrial chemicals. Chlorinated phenols are also a by-product of the paper manufacturing industry. Method 529 was developed to monitor for hexahydro-1,3,5-trinitro-1,3,5-triazine, also known as Royal Demolition Explosive (RDX), and an additional 13 explosives and related chemicals. Recent studies of groundwaters near military installations have identified RDX and other munitions as an emerging drinking water concern. Method 529 will be used in the next nationwide monitoring study to gather data on the occurrence of RDX and other explosives. NERL is currently working with OGWDW to develop three additional methods for chemicals listed on the CCL. These methods will be used to monitor perchlorate, organotins, alachlor-ESA and other acetanalide pesticide degradation products in a nationwide study beginning in 2006.

Rodgers, M.R., Vesper, S.J., Haugland, R.A., and Lye, D.J. The good, the bad and the ugly - determination of bacterial virulence using animal models and microarray technology. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Mark R. Rodgers

Abstract:

Rodgers, M.R., and Smallwood, A.W. Sensitivity of different aeromonas species to copper and silver. Presented at: American Society for Microbiology, Washington, DC, May 18-22, 2003. 5/18/2003

Contact: Mark R. Rodgers

Abstract: Aeromonas bacteria are common flora in surface and ground waters and are considered to be human pathogens. They can also be found in municipally treated drinking water, likely as a component of biofilms, as found in distribution system pipes and point of use water filters. It has been previously reported that Aeromonas bacteria are very sensitive to certain heavy metals, such as copper, found in treated drinking water and that this sensitivity affects the recovery of these bacteria from water samples. In addition, copper and silver ions are frequently employed as bactericidal agents in water filters to limit biofilm formation. Earlier studies on metal sensitivity of aeromonads did not investigate differences among species. We have undertaken the present study to investigate possible differences among Aeromonas species with regard to heavy metal sensitivities. Eleven Aeromonas species, including A. hydrophila, A. caviae, A. veronii biovar veronii and A. bestiarum (species most commonly encountered in both clinical and environmental samples) were used in the sensitivity studies. Heavy metal sensitivities of E. coli and Vibrio cholerae were also measured for comparison. Bacteria were exposed to varying concentrations of copper and silver ions in saline. Our copper sensitivity results support previous studies, indicating that Aeromonas bacteria, regardless of species, are more sensitive than E. coli to copper. V. cholerae displayed a similar copper sensitivity. Differences among Aeromonas species were seen however at exposure to 20 micromolar copper, providing a possible explanation for why certain species may be isolated more frequently than others from water. Silver, at concentration as low as 5 micromolar, had a pronounced effect on all strains tested, with the Aeromonas strains more sensitive than either E. coli or V. cholerae.

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Presented Published

Birkenhauer, J.M., and Rodgers, M.R. Identification and characterization of Aeromonas isolates from drinking water distribution systems. Presented at: American Society for Microbiology, Washington, DC, May 18-22, 2003. 5/18/2003

Contact: Mark R. Rodgers

Abstract: Members of the bacterial genus Aeromonas are commonly isolated from both fresh and salt waters worldwide and some are believed to cause infections in humans, including gastroenteritis and wound infections. Currently, aeromonads are on the United States Environmental Protection Agency's Contaminant Candidate List, and are suspected of contaminating drinking water distribution systems. Identification of aeromonads to the species level is difficult as new species, taxa, and biogroups continue to be proposed. In this study, we employ both metabolic and genomic fingerprinting identification methods to obtain an understanding of the occurrence and types of aeromonads in drinking water distribution systems in the US. Water samples were analyzed from 18 drinking water distribution systems across the US, eight of which were found to contain aeromonads. All colonies were isolated from ADA-V medium and were confirmed to be aeromonads as recommended in EPA Method 1605. Confirmed isolates, 212 in total, were then subjected both a Restriction Fragment Length Polymorphism (RFLP) analysis (Borrell et al, 1997) and to a carbon source utilization assay employing the BIOLOG microbial identification system. The BIOLOG microbial identification system offers a straightforward approach to identifying environmental microbes. However, we found that only after compiling our own database were we able to gain confidence in the system's ability to correctly identify each isolate. The RFLP analysis, while requiring much more time and technical skill, was able to give a more consistent identification of each isolate, with the exception to certain biotypes. Based on both the metabolic and genomic fingerprinting of these organisms we were able to identify several different biotypes, including A. hydrophila, A. bestiarum, and A. salmoncida from drinking water distribution systems. Since some of the species that were isolated have been implicated in human disease, the results from this study indicate that a more comprehensive survey of drinking water utilities is warranted to determine if aeromonads in drinking water pose a threat to public health.

Rodgers, M.R., Vesper, S.J., and Lye, D. The good, the bad and the ugly - determination of bacterial virulence using animal models and microarray technology. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Mark R. Rodgers

Abstract: In its Computational Toxicology Program, EPA/ORD proposes to integrate genomics and computational methods to provide a mechanistic basis for the prediction of toxicity of chemicals and the pathogenicity of microorganisms. The goal of microbiological water testing is to be able to determine with scientifically sound probabilities that the consumption of and/ or exposure to a given volume of water is safe. At present, the US EPA relies on indirect methods to determine the probabilities of the safety of drinking water, for example, the detection and enumeration of indicator microorganisms (e.g. E. coli). Although the concept of indicator organisms as a measure of risk has served to reduce water-borne disease significantly over the years, public health professionals generally agree that the presence of pathogens does sometimes escape detection and at an unknown frequency. Municipally treated drinking water is known to contain many bacteria, whose identity and potential human virulence is poorly understood. In some cases pathogens may not be culturable by our standard methods. In addition, polymicrobial disease agents may be present. Polymicrobial diseases are infections that result from multiple co-infecting organisms as opposed to a single microorganism. Application of computational-toxicologically based DNA microarrays offers a totally new approach to address the issue of water-borne pathogens. There are many potential human pathogens in potable water, some identified and some not yet identified. In order to cause disease, a pathogen must possess "virulence factors" that interact with a host cell to cause damage that results in disease. The interaction with a host cell results in a change in the metabolism and thus the messenger RNAs (mRNAs) that are produced by the human cell(s). These changes can be monitored using Human DNA microarrays which will reveal whether a pathogen is present and whether it is virulent. Ultimately, we will build a database of these mRNA responses to more and more known pathogens and the virulence factors associated with inducing the mRNAs. It may be possible to then use this database to predict the potential pathogenicity of unknown or non-culturable pathogens based on their producing similar mRNA responses found in known pathogens. Some animal experiments may then be used to confirm these predictions. However, ultimately this type of analysis should dramatically reduce the EPA's need for animals in experimentation.

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Presented Published

Rodgers, M.R., and Smallwood, A.W. Sensitivity of different aeromonas biotypes to copper and silver. Presented at: American Society for Microbiology, Washington, DC, May 18-22, 2003. 5/18/2003

Contact: Mark R. Rodgers

Abstract:

Birkenhauer, J.M., and Rodgers, M.R. Identification and characterization of aeromonas isolates from drinking water distribution systems. Presented at: American Society for Microbiology, Washington, DC, May 18-22, 2003. 5/18/2003

Contact: Mark R. Rodgers

Abstract:

Lye, D.J. Pathogenicity of biofilm bacteria. Presented at: USEPA's Research on Micoorganisms in Drinking Water Workshop, Cincinnati, OH, August 5-7, 2003. 8/5/2003

Contact: Dennis J. Lye

Abstract: There is a paucity of information concerning any link between the microorganisms commonly found in biofilms of drinking water systems and their impacts on human health. For bacteria, culture-based techniques detect only a limited number of the total microorganisms associated with biofilms. The possibility of unknown opportunistic pathogens occurring in potable water and biofilms within drinking water systems still exists but it is unlikely that pathogenic microorganisms will be found using individual in vivo culture-based techniques or by screening large numbers of isolates using the currently available in vitro virulence tests. A combination of molecular-based techniques and animal-exposure studies will provide the information necessary to fully characterize the pathogenicity of microorganisms commonly associated with biofilms.This is an abstract of a proposed presentation and does not necessarily reflect the United States Environmental Protection Agency (EPA) policy. The actual presentation has not been peer reviewed by EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Haugland, R.A., and Vesper, S.J. New laboratory methods - DNA immunology. Presented at: Expert Indoor Mycology Workshop, Asilomar, CA, July 31, 2003. 7/31/2003

Contact: Richard A. Haugland

Abstract: Indoor fungi present potential, although as yet not fully defined, health risks to the occupants of heavily contaminated buildings due to their production of allergens, and a wide range of mycotoxins. A better understanding of the health risks posed by these organisms will require accurate, quantitative estimates of the occurrence of individual species or groups of species with common allergenic and/or biologically active compound production characteristics as well as the development of mechanistic biomarkers of human exposure. The same culture-based, microscopic and chemical methods have been used for decades with few improvements for identifying fungi and measuring their occurrence in environmental and clinical samples. Molecular technologies now offer the opportunity to provide more rapid, accurate and standardized measurements of fungi and fungal biomarkers. This presentation will review recent nucleic acid and immunology based methods that have been developed for the detection of indoor fungi with emphasis on real time PCR and ELISA methods, developed by the U.S. EPA, Office of Research and Development, for quantifying the occurrence of and human exposure to these organisms.

Haugland, R.A., Brenner, K.P., Dufour, A.P., and Siefring, S.D. The need for speed - rapid methodologies to determine bathing beach water quality. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Richard A. Haugland

Abstract:

Haugland, R.A. Rapid measurement of bacterial fecal indicators in surface waters by quantitative polymerase chain reaction (QPCR) analysis. Presented at: Rapid Microbiological Measurement Method Workshop, Monterey, CA, May 14-16, 2003. 5/14/2003

Contact: Richard A. Haugland

Abstract: Current methods for determining fecal contamination of recreational waters rely on the culture of bacterial indicators and require at least 24 hours to determine whether the water is unsafe for use. By the time monitoring results are available, exposures have already occurred. New methods are needed that will allow near real-time determination of water quality, such that public notifications can be made and hazardous exposures avoided. With assistance from the U.S. Geological Survey Laboratory in Porter IN, the U.S. EPA, National Exposure Research Laboratory has conducted a two year pilot study to evaluate the use of quantitative PCR (QPCR) analysis for measuring water-borne fecal indicator microorganisms at two recreational beaches on Lake Michigan.A rapid, simple and generally applicable method for the recovery of total DNA from various microorganisms in water samples has been developed. The method involves filtration of water samples on polycarbonate membrane filters and disruption of the collected cells directly on the membranes by glass bead milling. Recovered DNAs are subjected to QPCR analysis using the TaqManTM PCR product detection system in a real time PCR product detection instrument. Procedures have also been developed for the use of cycle threshold (CT) values generated by the instrument to enumerate cells in the water samples. The approach is based on the comparative cycle threshold (CT) method, which employs an arithmetic formula to determine target sequence quantities in DNA extracts from test samples relative to those in similarly-prepared DNA extracts from calibrator samples containing a known quantity of target organism cells. Assay CT values for a DNA sequence from an exogenous reference organism, added in equal quantities to both the test and calibrator samples before extraction, are used to normalize results for differences in the amount of total DNA added to each reaction (e.g., caused by differences in DNA extraction efficiency between samples) or to signal potential PCR inhibition in test samples. The entire analysis process can be performed in approximately two to three hours.Target DNA sequences for QPCR detection in the pilot study included the large subunit ribosomal RNA gene of Enterococcus spp. and the small subunit ribosomal RNA gene of Bacteroides spp. Tests with pure cultures of representative species within these two genera gave extrapolated detection limits of approximately two cells per sample for the Enterococcus assay and 25 cells per sample for the Bacteroides assay. Tests on a subset of the Lake Michigan water samples spiked with ~1000 cells of Enterococcus cells gave an overall mean value of 0.96 for the ratio of measured to added cells and a 95% occurrence range for individual sample ratios of ~0.3 to 3, based on analyses of three replicates of each sample. Mean QPCR-measured quantities of native enterococci in the 100 ml water samples ranged from less than 10 to ~1000 cells whereas mean quantities of Bacteroides ranged from less than 100 to ~100,000 cells. DNA extracts of the water samples were routinely diluted 10-fold prior to analysis to eliminate the effects of PCR inhibitors. The distributions of QPCR-measured cell quantities of both Enterococcus and Bacteroides in the water samples paralleled those of culturable enterococci measured in corresponding water samples by the currently accepted mEI filter plating method. These results indicate that the QPCR method has the potential to detect a broad range of fecal indicator densities in recreational water samples. Past findings of a correlation between the quantities of culturable enterococci in water samples and illness rates among bathers have provided the basis for establishing recreational water quality guidelines. New studies will be initiated this summer to establish whether similar correlations exist between fecal indicator measurements by QPCR and other rapid methods and rates of illness among bathers.

Haugland, R.A. The need for speed-rapid methodologies to determine bathing beach water quality. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Richard A. Haugland

Abstract: Current methods for determining fecal contamination of recreational waters rely on the culture of bacterial indicators and require at least 24 hours to determine whether the water is unsafe for use. By the time monitoring results are available, exposures have already occurred. New methods are needed that will allow near real-time determination of water quality, such that public notifications can be made and hazardous exposures avoided. The U.S. EPA, National Exposure Research Laboratory (NERL) has evaluated several new antibody and molecular-based approaches for obtaining timely measurements of recreational water quality within a two-three hour time period. These approaches include: 1) flow cytometric detection of indicator organisms using fluorescently labeled antibody-paramagnetic bead complexes; 2) antibody capture and fiber optic detection of indicator organisms; and 3) quantitative polymerase chain reaction amplification of nucleic acids from specific fecal indicator organisms. These methods will be used in a joint study by NERL, the National Health and Environmental Effects Laboratory and the Centers for Disease Control to develop new water quality-health effects relationships that can be used for establishing scientifically defendable guidelines for recreational waters.The objectives of this research are: (1) to obtain in a timely manner water quality data using the new rapid, state-of-the-art methods and the new EMPACT monitoring protocol in conjunction with epidemiological studies that will produce water quality-health data; and (2) to provide the information to the Office of Water so they can promulgate new state and/or federal guidelines and limits for water quality indicators of fecal contamination so that beach managers and public health officials can alert the public about the potential health hazards before exposure to unsafe water can occur.

Fout, G.S. Detection by PCR of human enteric viruses concentrated from large volumes of water. Presented at: EPA Workshop for Development of Protocols for Reliable Genetic Methods for Viruses for Use in EPA's Drinking Water Program, Cincinnati, OH, January 15-16, 2003. 1/15/2003

Contact: G. shay Fout

Abstract: Viruses are recovered and concentrated from water by passage through a positively charged cartridge filter. Following virus elution from the cartridge filter with beef extract and concentration of the beef extract solution, viruses are usually assayed by cell culture. However, cultural methods are too time consuming and expensive for routine use and many of the viruses that cause waterborne disease are either very difficult to culture or cannot be cultured. Rapid polymerase chain reaction (PCR) methods have been developed to overcome these problems. While PCR methods are rapid and can detect all the virus groups known to cause waterborne disease, the methods have several unique problems that can cause false negative and false positive results. The presence of potent environmental inhibitors of PCR that are co-concentrated along with viruses during sample processing can result in false negative results. In addition, aerosols containing previously amplified products can cause false-positive results. The quality control measures needed to prevent these problems are much more complex than those used in traditional microbiology or chemistry laboratories. The number and types of environmental inhibitors vary among different water types and even within a single water type. Thus PCR methods must be designed to remove as many inhibitors as possible from different water types without affecting virus recovery. A multiplex PCR method was developed at the U.S. EPA to measure the occurrence of enteroviruses, reoviruses, rotaviruses, hepatitis A virus and Norwalk virus in water. The method uses a celite-based elution/reconcentration procedure. It results in concentrating a portion of the filter eluate by greater than 800 fold while providing a 74% recovery of poliovirus. Although the method was very effective, false negative and positive results still occur. In one study false negative results were observed in 14% of the samples and false positive results in 6%. The method and appropriate quality controls will be described.

Fout, G.S. The key viral players. Presented at: EPA Workshop for Development of Protocols for Reliable Genetic Methods for Viruses for Use in EPA's Drinking Water Program, Cincinnati, OH, January 15-16, 2003. 1/15/2003

Contact: G. shay Fout

Abstract: A number of different types of human enteric viruses cause waterborne outbreaks when individuals are exposed to contaminated drinking and recreational waters. Members of the enterovirus group cause numerous diseases, including gastroenteritis, encephalitis, meningitis, myocarditis, temporary paralysis and perhaps diabetes and chronic fatigue syndrome. Hepatitis A and more recently hepatitis E have caused large waterborne hepatitis outbreaks. The second leading cause of illness in the United States is acute nonbacterial gastroenteritis. This disease results from infection of susceptible individuals with members of the Caliciviridae, Astroviridae, Reoviridae and Adenoviridae families. The characteristics of these enteric virus groups will be discussed.

Grimm, A.C., Fout, G.S., Cashdollar, J.L., and Williams, F.P. Development of a molecular method to detect astrovirus. Presented at: American Society for Virology Annual Meeting, Davis, CA, July 12-16, 2003. 7/12/2003

Contact: G. shay Fout

Abstract: Astrovirus is a common cause of gastroenteritis that has been determined to be responsible for several outbreaks. Since astrovirus can be waterborne, there is interest in testing environmental water for astrovirus and we have developed a sensitive RT-PCR assay that is designed to detect all known astrovirus. When tested, this assay was able to detect strains from all eight serotypes. In addition, an internal control was developed so that it will be possible to determine if the sample being tested contains PCR inhibitors. Most probable number analysis determined that when amplified with the developed assay, a single DNA molecule of the internal control could be detected if inhibitors were not present. The assay was successfully adapted to real-time PCR and this method was then used for integrated cell culture RT-PCR detection of live virus. The methods were successfully used to detect astrovirus present in clinical samples and spiked water samples.

Fout, G.S. EPA methods for virus detection in water. Presented at: University of Surrey - Site Visit, Guildford, UK, March 28, 2003. 3/28/2003

Contact: G. shay Fout

Abstract: A number of different types of human enteric viruses cause waterborne outbreaks when individuals are exposed to contaminated drinking and recreational waters. Members of the enterovirus group cause numerous diseases, including gastroenteritis, encephalitis, meningitis, myocarditis, temporary paralysis and perhaps diabetes and chronic fatigue syndrome. Hepatitis A and more recently hepatitis E have caused large waterborne hepatitis outbreaks. The second leading cause of illness in the United States is acute nonbacterial gastroenteritis. This disease results from infection of susceptible individuals with members of the Caliciviridae, Astroviridae, Reoviridae and Adenoviridae families. The characteristics of these enteric virus groups will be discussed. Viruses are recovered and concentrated from water by passage through a positively charged cartridge filter. Following virus elution from the cartridge filter with beef extract and concentration of the beef extract solution, viruses are usually assayed by cell culture. However, cultural methods are too time consuming and expensive for routine use and many of the viruses that cause waterborne disease are either very difficult to culture or cannot be cultured. Rapid polymerase chain reaction (PCR) methods have been developed to overcome these problems. While PCR methods are rapid and can detect all the virus groups known to cause waterborne disease, the methods have several unique problems that can cause false negative and false positive results. The presence of potent environmental inhibitors of PCR that are co-concentrated along with viruses during sample processing can result in false negative results. In addition, aerosols containing previously amplified products can cause false-positive results. The quality control measures needed to prevent these problems are much more complex than those used in traditional microbiology or chemistry laboratories. The number and types of environmental inhibitors vary among different water types and even within a single water type. Thus PCR methods must be designed to remove as many inhibitors as possible from different water types without affecting virus recovery. A multiplex PCR method was developed at the U.S. EPA to measure the occurrence of enteroviruses, reoviruses, rotaviruses, hepatitis A virus and Norwalk virus in water. The method uses a celite-based elution/reconcentration procedure. It results in concentrating a portion of the filter eluate by greater than 800 fold while providing a 74% recovery of poliovirus. Although the method was very effective, false negative and positive results still occur. In one study false negative results were observed in 14% of the samples and false positive results in 6%. The method and appropriate quality controls will be described.

Fout, G.S. The application of emerging technologies to virus detection in water. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: G. shay Fout

Abstract: Human enteric viruses belonging to many different viral genera cause waterborne disease when susceptible individuals are exposed to contaminated drinking and recreational waters. Diseases resulting from infection with these viruses include gastroenteritis, hepatitis, encephalitis, meningitis, myocarditis, temporary paralysis and possibly diabetes. Symptoms are often mild, but can be severe in sensitive target groups, such as the very young and elderly. It is important that methods based upon emerging technologies are able to detect many of the viral groups that cause waterborne disease. Specific virus methods, based upon the polymerase chain reaction (PCR), have been developed by the National Exposure Research Laboratory (NERL). While PCR methods are rapid and can detect all the virus groups known to cause waterborne disease, the methods have several unique problems that require a much higher level of quality assurance than that used in traditional microbiology or chemistry laboratories. A quality assurance guide for using PCR methods in support of EPA's mission is being developed through a collaborative partnership of NERL and the Technical Support Center (TSC) of the Office of Ground Water and Drinking Water. This QA guide is a key outcome of a recent, jointly sponsored workshop on PCR methods. NERL's PCR methods were originally developed as part of a national groundwater survey that was conducted through the partnering of NERL, TSC and the American Water Works Association Research Foundation. In order to determine whether the methods would detect viruses in surface waters and test the level of quality assurance that had been developed, further collaboration was sought with another partner, the U.S. Geological Survey. The studies on ground and surface waters demonstrated that the methods could be used on a majority of water types covering most of the conditions typically found in the U.S. Methods were further tested in collaborative studies to determine the cause of two independent waterborne outbreaks of gastroenteritis in Wyoming during 2001. These studies involved personnel from Wyoming, the Centers for Disease Control, EPA's Region VIII and the University of North Carolina. In both cases NERL's methods successfully identified the agent in water that was responsible for the outbreak. NERL's collaborative efforts to develop and evaluate emerging virus detection methodology have clearly demonstrated the usefulness of the partnering process.

Fout, G.S., Grimm, A.C., Dahling, D.R., Cashdollar, J.L., Newport, C., Parshionikar, S., and Willian-True, S. Rapid virus detection in water. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: G. shay Fout

Abstract:

Brenner, K. Environmental monitoring for public access and community tracking (EMPACT) program microbiological monitoring of recreational water. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Kristen P. Brenner

Abstract: Current Environmental Protection Agency (EPA) recommended microbiological monitoring practices for bathing beach water quality were suggested in 1968, as a part of the fecal coliform guideline developed by the Federal Water Pollution Control Administration. The guideline stated that the geometric mean of the fecal coliform counts from five water samples taken over a thirty-day period would be used to determine the beach water quality. This level would be compared with an established limit beyond which the risk of illness was unacceptable. Although EPA has developed much better health guidelines for bathing beach waters that were recommended to the states in 1986, the old methods for monitoring continue to be used by many states and local public health authorities. This approach does not provide timely, accurate information for risk managers or the public, nor does it provide results that are easily interpreted. This shortcoming can be overcome by developing a statistically valid monitoring protocol that takes into account the sampling and environmental factors that vary considerably and, hence, contribute to the uncertainty on how and when to sample and how to interpret the results. This research study examined five representative beaches from various sections of the United States in depth. The beaches were selected to obtain data on a variety of pollution sources, population density, type of swimming water (fresh or marine), and the type of beach (large coastal beaches, small lake or impoundment beaches, river recreational areas). An appropriate sampling design was developed to account for variation associated with spatial factors, e.g., depth of water, length of the beach and its distance from shore to permissible swimming limits, and temporal factors, such as hourly, daily and seasonal variation. By considering these factors, more appropriate site-specific monitoring protocols can be developed that are based on sound science and will result in better protection of the public health. The EMPACT project was a collaborative effort between a team of NERL-Cincinnati scientists, two contractors, Lockheed Martin and Battelle, and the collaborating EMPACT cities and laboratories. In addition, outside experts helped with the planning process through their involvement in a Data Quality Objectives Workshop, provided input during the statistical analysis, and participated in a final workshop to review the statistical analysis of the study data. The study report will be used by the Office of Water to develop official monitoring guidelines and a mechanism for translating technical monitoring data into a simple system the public can use to make personal decisions about risks associated with swimming activities.

Brenner, K.P., Dufour, A.P., and Wymer, L.J. Environmental monitoring for public access and community tracking (EMPACT) program microbiological monitoring of recreational water. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Kristen P. Brenner

Abstract:

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Presented Published

McDaniels, A.E., Rice, E.W., Tally, R.A., Wymer, L.J., and Stelma, Jr., G.N. A comparison of three assay procedures for determining chlorine inactivation of waterborne pathogenic bacteria. Presented at: 103rd General Meeting American Society for Microbiology, Washington, DC, May 18-22, 2003. 5/18/2003

Contact: Audrey E. Mcdaniels

Abstract: One criterion on which chlorine treatment of water may be based is the concentration (C) in mg/l multiplied by the time (t) in min of exposure or Ct values. We compared different Ct values on waterborne pathogenic bacteria by cultural assay for viability and 2 assays that measured metabolic activity. Total counts were measured by 4'6-diamidino-2-phenylindole dihydrochloride (DAPI). Metabolic activity was based on the presence of esterase as detected by a modified fluorescein diacetate fluorophore designated V6 and respiration by the fluorophore 5-cyano-2,4-ditoyl tetrazolium chloride (CTC). V6 results were obtained using a solid phase cytometer and counts were validated with a microscope. CTC and DAPI results were obtained by applying a mathematical formula to determine microscopic counts of cells per ml. The bacteria included Escherichia coli 0157:H7, Legionella pneumophila and Helicobacter pylori. Each bacterium was added to 3 separate chlorine demand free water samples. Three different Ct values per sample were examined at 5oC and pH 7.0. Counts were obtained before and after chlorine treatment. No significant differences were found between the DAPI controls and the corresponding chlorine treated samples. In the Chick-Watson theory of disinfection the rate of die-off of microorganisms is hypothesized to be proportional to the number of organisms remaining and a power function of the disinfectant concentration. This theory was shown to be reasonable for E. coli 0157:H7, L. pneumophila and H. pylori where no plate growth or CTC counts were found after exposure to Ct ranges of 7 to 95. V6 counts for these 3 treated bacteria were present but as Ct values increased, counts were significantly reduced. After chlorine treatment each bacterium decreased by at least 2 to 3 log10 for all assays. V6 activity persisted when other assays were negative and would thus be more apt to indicate any lingering activity within the cells. It may be a conservative indicator of metabolic activity within bacteria.

Vesper, S.J. A revolution in mold identification and enumeration. Presented at: Building Environment Council of Ohio Spring Conference, Columbus, OH, April 10, 2003. 4/10/2003

Contact: Stephen J. Vesper

Abstract: More than 100 assay were developed to identify and quantify indoor molds using quantitiative PCR (QPCR) assays. This technology incorporates fluorigenic 5' nuclease (TaqMan.) chemistry directed at the nuclear ribosomal RNA operon internal transcribed spacer regions (ITS1 or ITS2). The assays varied in specificity from species to closely related groups of species, subject to the amount of nucleotide sequence variation in the different organisms. Estimated conidia detection limits ranged from less than one to several hundred per sample for the different assays, using a previously reported glass bead milling and glass filter purification DNA extraction method. The precision, accuracy and sensitivity of the method were determined from analyses of replicate mixed conidia suspensions and different dust samples spiked with known quantities of target organisms.

Ware, M.W., Schaefer, III, F.W., Rice, E.W., and Hayes, S.L. Inactivation of giardia muris by low pressure ultraviolet light. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Michael W. Ware

Abstract:

Ware, M.W., Schaefer, III, F.W., Hayes, S.L., and Rice, E.W. Inactivation of Giardia muris by low pressure ultraviolet light. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Michael W. Ware

Abstract:

Grimm, A.C., Cashdollar, J.L., Williams, F.P., and Fout, G.S. Development of a molecular method to identify astrovirus in water. Presented at: USEPA Microorganisms in Drinking Water Workshop, Cincinnati, OH, August 5-7, 2003. 8/5/2003

Contact: Ann Grimm

Abstract: Astrovirus is a common cause of gastroenteritis that has been determined to be responsible for several outbreaks. Since astrovirus can be waterborne, there is interest in testing environmental water for astrovirus. We have developed a sensitive reverse transcription-polymerase chain reaction (RT-PCR) assay that is designed to detect all known astrovirus strains. The assay was based on a primer set that contained multiple upper and lower primers as well as multiple probes. This would allow for amplification of all of the known strains of astrovirus using a single reaction. When tested, this assay was able to detect strains from all eight serotypes. In addition, an internal control was developed so that it will be possible to determine if the sample being tested contains PCR inhibitors. Most probable number analysis determined that when amplified with the developed assay, a single DNA molecule of the internal control could be detected if inhibitors were not present. The assay was successfully adapted to real-time PCR and this method was then used for integrated cell culture/RT-PCR detection of infectious virus. The methods were successfully used to detect astrovirus present in clinical samples and spiked water samples.A simple, sensitive method for detecting all known astrovirus strains has been developed that can be used to detect this virus in water. This assay will be field tested by analyzing environmental water samples.

Grimm, A.C., Cashdollar, J.L., Williams, F.P., and Fout, G.S. Development of a molecular method to identify astrovirus in water.. Presented at: Science and Mission Club, Cincinnati, OH, June 4, 2003. 6/4/2003

Contact: Ann Grimm

Abstract: Astrovirus is a common cause of gastroenteritis that has been determined to be responsible for several outbreaks. Since astrovirus can be waterborne, there is interest in testing environmental water for astrovirus and we have developed a sensitive RT-PCR assay that is designed to detect all known astrovirus. When tested, this assay was able to detect strains from all eight serotypes. In addition, an internal control was developed so that it will be possible to determine if the sample being tested contains PCR inhibitors. Most probable number analysis determined that when amplified with the developed assay, a single DNA molecule of the internal control could be detected if inhibitors were not present. The assay was successfully adapted to real-time PCR and this method was then used for integrated cell culture RT-PCR detection of infectious virus. The methods were successfully used to detect astrovirus present in clinical samples and spiked water samples.

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Presented Published

Furlong, E.T., Ferrer, I., Glassmeyer, S., Cahill, J.D., Zaugg, S.D., Werner, S.L., Kolpin, D.W., and Kryak, D.D. Distribution of organic wastewater contaminants between water and sediment in surface waters of the United States. Presented at: National Groundwater Association 3rd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, Minneapolis, MN, March 19-21, 2003. 3/19/2003

Contact: Susan Glassmeyer

Abstract: Trace concentrations of pharmaceuticals and other organic wastewater contaminants have been determined in the surface waters of Europe and the United States. A preliminary report of substantially higher concentrations of pharmaceuticals in sediment suggests that bottom sediment may be an important reservoir of pharmaceuticals discharged to surface water. The U.S. Geological Survey undertook a systematic examination of bottom sediment collected from a dozen sites across the United States, in order to determine the range of compositions and concentrations of pharmaceuticals and other organic wastewater contaminants present in those sediments. Composite sediment samples were collected at one point upstream and two points downstream of a point source discharge. Surface water samples were collected concurrently at the same sites, as well as at the point discharge. The samples were shipped by overnight express and water samples were analyzed within 48 hours of receipt. Sediments were held frozen until extraction and analysis. Pharmaceuticals were extracted from filtered water samples by solid-phase extraction and the concentrated extract analyzed by high-performance liquid chromatography/mass spectrometry (HPLC/MS). Other organic wastewater contaminants were extracted from whole-water samples by continuous liquid-liquid extraction followed by gas chromatography/mass spectrometry (GC/MS; Kolpin and others, 2002). Sediments were extracted by accelerated solvent extraction and the extracts analysed by HPLC/MS (pharmaceuticals) or GC/MS (other wastewater constituents).Preliminary results indicate that a wide range of pharmaceuticals and other wastewater constituents are present in sediments at concentrations substantially higher than in surface water. For example, caffeine, a highly water soluble contaminant, has estimated partitioning coefficient of 0.85, suggesting that concentrations should be higher in water than in sediment. However, the ratio observed for caffeine in environmental samples ranges between 4 and 136. Similarly, the predicted partitioning coefficient for trimethoprim is 8.2, while the observed partitioning coefficients range between 17 and 1,100. Also, as water and sediment concentrations increase, the observed partitioning coefficient more closely approaches the predicted partitioning coefficient. Further, pharmaceuticals were not detected in pore-water samples, suggesting that pharmaceuticals are intimately associated with the sediment phase. Taken together, these data suggest that sediments are potentially important reservoir for sequestering pharmaceuticals in surface water systems. Determining the mechanisms of association between water-soluble pharmaceuticals and sediment will be an important new area of research.

Glassmeyer, S., Ferrer, I., Furlong, E.T., Cahill, J.D., Zaugg, S.D., Werner, S.L., Kolpin, D.W., and Kryak, D.D. Transport of chemical and microbial contaminants from known wastewater discharges. Presented at: National Groundwater Association 3rd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, Minneapolis, MN, March 19-21, 2003. 3/19/2003

Contact: Susan Glassmeyer

Abstract: The quality of drinking and recreational water is currently ascertained using indicator bacteria, such as Escherichia coli and fecal enterococci. However, the tests to analyze for these bacteria require a considerable length of time to complete, and do not discriminate between human and animal fecal material sources. One solution to these problems is to use chemicals that are commonly found in human wastewater as supplementary tracer compounds. The chemicals have the advantage of requiring shorter analysis times, and they can be chosen to be human specific markers. For this project, we focused on a variety of compounds to determine their efficacy as chemical indicators of human fecal contamination. This list included compounds that are produced and excreted by humans (coprostanol, urobilin), that are consumed and pass easily through humans (pharmaceuticals and caffeine), and that are associated with humans and deposited into the combined graywater/ blackwater household septic waste stream (surfactants). Stream samples were collected upstream, at the point of discharge, and at two points downstream from wastewater treatment facilities at ten locations. This longitudinal sampling scheme was developed to determine the persistence of the compounds in streams. Compounds that are quickly removed or degraded may not be persistent enough to serve as tracers; those that are too recalcitrant would similarly not be suitable as they would be present after the pathogens have been eliminated. To estimate the desired duration, the water samples were analyzed for E. coli and fecal enterococci in addition to the suite of chemicals. For the chemicals, the water samples were extracted using either solid phase extraction (for the pharmaceuticals) or liquid-liquid extraction (for the other wastewater contaminants) and were analyzed using either high-performance liquid chromatography/mass spectrometry (HPLC/MS; pharmaceuticals) or gas chromatography/mass spectrometry (GC/MS; other wastewater contaminants; Kolpin et al., 2002). To analyze for the microbial contaminants, three aliquots of water (100, 10, and 1 mL) were filtered through cellulose disks and placed on modified mTEC (E. coli,) or mEI (enterococci) media and incubated (USEPA, 2000).

Glassmeyer, S., Kelty, C.A., and Santo Domingo, J. Fingerprinting of fecal enterococci by matrix assisted laser desorption ionization mass spectrometry. Presented at: American Society of Microbiology, Washington, DC, May 18-22, 2003. 5/18/2003

Contact: Susan Glassmeyer

Abstract: The fecal enterococci group has been suggested as an indicator of fecal contamination in freshwater and marine water systems and as a potential target for bacterial source tracking of fecal pollution. While many studies have described the diversity of enterococci in environmental waters, most of these studies have relied on biochemical characteristics that can not discriminate between some of the different species of fecal enterococci. In this study, we evaluated the use of whole cell protein profiles using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as a tool to identify different enterococci species. Seven enterococci species were used in this study, namely, Enterococcus faecalis, E. faecium, E. durans, E. gallinarum, E. avium, E. mundtii, and E. casseliflavus. Analysis of protein spectral profiles of masses lower than 2000 mass-to-charge (m/z) were identical between all enterococci species tested suggesting that these might be characteristic of the enterococci group. While many peaks were also shared among the different enterococci species in the 4,000 to 11,000 m/z range, each species showed distinctive peaks, primarily in the 6,000 to 7,000 m/z region. When environmental isolates were tested, the signature peaks were observed in many of the different isolates, suggesting that these peaks could be used for species identification. Sequence analysis of the environmental isolates 16S rDNA confirmed the identity of the strains tested. The results from this study indicate that the analysis of whole cell by MALDI generates a protein profile which can be used for the rapid identification of fecal enterococci environmental isolates. Since not all environmental isolates that belong to the same species had an identical protein profile, these results suggest that MALDI may also be used to discriminate between different strains of the same enterococci species.

Glassmeyer, S., Ferrer, I., Furlong, E.T., Cahill, J.D., Zaugg, S.D., Werner, S.L., Meyer, M., Kolpin, D.W., and Kryak, D.D. Transport of chemical and microbial contaminants from known wstewater discharges: potential chemical indicators of human fecal contamination. Presented at: U.S. EPA's Research on Microorganisms in Drinking Water Workshop, Cincinnati, OH, August 5-7, 2003. 8/5/2003

Contact: Susan Glassmeyer

Abstract: The quality of drinking and recreational water is currently ascertained using indicator bacteria, such as Escherichia coli and fecal enterococci. However, the tests to analyze for these bacteria require 24 to 48 hours to complete, and do not discriminate between human and animal fecal material sources. One solution to these problems is to use chemicals that are commonly found in human wastewater as supplementary tracer compounds. The chemicals have the advantage of requiring shorter analysis times (3-4 hours), and a suite of human specific markers can be selected that are unique to human wastewater. For this project, compounds includes those that are produced and excreted by humans (e.g. coprostanol), that are consumed and pass easily through humans (e.g. pharmaceuticals and caffeine), and that are associated with humans and deposited into the combined graywater/ blackwater household septic waste stream (e.g. surfactants). At ten wastewater treatment facilities, a treated effluent sample, as well as surface water samples from upstream, and at two successive points downstream from the facility were collected. This longitudinal sampling scheme was used to determine the persistence of the target compounds in streams. Compounds that are quickly removed or degraded may not be persistent enough to serve as tracers; those that are too recalcitrant would similarly not be suitable as they would be present after the pathogens have been eliminated. To estimate the environmental persistence of pathogens, the water samples were analyzed for E. coli and fecal enterococci in addition to the suite of chemicals being measured. For chemical analysis, the water samples were extracted using either solid phase extraction (for the pharmaceuticals) or liquid-liquid extraction (for the other wastewater contaminants) and were analyzed using either high-performance liquid chromatography/mass spectrometry (HPLC/MS; pharmaceuticals) or gas chromatography/mass spectrometry (GC/MS; other wastewater contaminants). The concentration of microbial indicators was determined using modified mTEC (E. coli) or mEI (enterococci) media. Of the 114 chemical analytes investigated in this project, more than 80 were found in at least one sample. While most concentrations were in the range of 0.1 to 1.0 mg/ L, in some of the more highly contaminated samples, concentrations were in the range of 5-20 mg/ L. The concentrations of the majority of the chemical compounds present in the samples generally followed the expected trend: they were either non-existent or at only trace levels in the upstream samples, had their maximum values in the wastewater effluent samples, and then declined in the two downstream samples. However, at most locations, there were indicator bacteria in the upstream samples, illustrating some of the difficulty in using bacteria to monitor water quality. This work indicates that these human wastewater constituents do have utility as tracers of human wastewater discharge. However, until the behavior of these chemical analytes is evaluated in a rigorous epidemiological study, their true potential as chemical indicators of human fecal contamination will not be determined. To begin this assessment, samples are currently being analyzed as part of the National Epidemiological and Environmental Assessment of Recreational Water Study, which should determine if there is a correlation between concentration of any of the chemicals and incidence of illness.

Cashdollar, J.L., Parshionikar, S., Newport, C., Willian-True, S., Dahling, D.R., and Fout, G.S. Methods used to analyze a norovirus outbreak. Presented at: USEPA's Research on Micoorganisms in Drinking Water, Cincinnati, OH, August 5-7, 2003. 8/5/2003

Contact: Jennifer Cashdollar

Abstract: Project Goals and Objectives:To isolate and identify the viral agents in well water samples associated with two outbreaks of acute gastroenteritis reported to the Wyoming Department of Health in February 2001 and October 2001. To isolate and identify the viral agents in patient stool samples and to determine the link between water consumption and illness.Approach: The project had a three way approach:An epidemiological investigation was performed to identify any common routes of exposure among those afflicted with gastroenteritis. An environmental survey was done of the premises involved in each outbreak to determine possible sources of contamination. Laboratory analysis was performed on well water samples for coliform and viral detection using RT-PCR and DNA sequencing. Stool samples were also analyzed for the presence of noroviruses.Preliminary Findings:Epidemiological studies revealed a close association between water consumption and illness. Environmental surveys in both outbreaks determined that the water supply was vulnerable to fecal contamination. Well water samples in both cases were positive for coliforms, and RT-PCR and DNA sequencing revealed noroviruses as the causative agents of acute gastroenteritis. Significance of Findings:This investigation demonstrates that EPA's viral concentration and molecular methods in conjunction with epidemiological and environmental analysis are very useful in outbreak studies.Next Steps:The methods used in this study can be performed in most laboratories with trained personnel and appropriate equipment, which would allow for routine monitoring of enteric viruses in drinking water, thus preventing any future outbreaks from occurring.

Bernard, C.E., Morgan, J.N., and Melnyk, L.J. Detection of toxicant(s) on building surfaces following chemical attack. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Craig E. Bernard

Abstract:

Bernard, C.E., Morgan, J.N., and Melnyk, L.J. Detection of toxicant(s) on building surfaces following chemical attack. Presented at: Science Forum 2003, Washington, DC, May 5-7, 2003. 5/5/2003

Contact: Craig E. Bernard

Abstract: A critical step prior to reoccupation of any facility following a chemical attack is monitoring for toxic compounds on surfaces within that facility. Low level detection of toxicant(s) is necessary to ensure that these compounds have been eliminated after building decontamination. This requires developing a sensitive, rapid, and systematic sampling protocol that can be used to measure low levels of toxic compounds on building surfaces. Although the military and other organizations have performed research on traditional chemical weapons, many industrial chemicals may be used as nontraditional chemical agents. For example, many pesticides are readily available to almost anyone, including potential terrorists. Although pesticides are typically not as potent as traditional chemical weapons, the release of such compounds into a building would certainly cause panic to the occupants and would present a chronic health concern if not properly removed. However, limited research has been performed with respect to such misuse of these compounds.Since the compounds that might be encountered in a chemical attack are diverse, the surface sampling protocol will be developed using a variety of industrial chemicals including pesticides. The selection of these compounds will be based on factors such as amounts manufactured worldwide, usage, and availability. Some classes of pesticides (e.g., organophosphate insecticides) will also be used because of their structural similarity to compounds that have been developed as chemical weapons. The procedure also needs to be applicable to a variety of building surfaces, such as walls, floors, ceilings, office furniture, and duct work. Therefore, different types of building surfaces will be contaminated with the study compounds at known concentrations and subsequently sampled. Surface sampling will consist of wipes with organic solvent moistened gauze pads, which were developed from previous EPA-NERL sponsored pesticide surface monitoring studies. Analytical methods will be optimized for detecting and measuring the study compounds in extracts from the gauze wipes. This project will result in the production of protocols for conducting surface wipes to collect toxicant(s) from contaminated building surfaces, as well as the detection and quantification of compounds captured in these surface wipes. Since the selected toxic compounds in this study will encompass a wide range of physical and chemical properties, the techniques could be applied to a wide variety of industrial chemicals and other chemical agents. These protocols and analytical procedures will be available for use by EPA and its partners in an emergency response to chemical terrorism.

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Presented Published

Bernard, C.E., Melnyk, L.J., and Berry, Jr., M.R. Transfer efficiencies of household pesticides from surfaces to foods. Presented at: 40th Annual AFDOSS Florida Pesticide Residue Workshop, St. Petersburg, FL, July 20-23, 2003. 7/20/2003

Contact: Craig E. Bernard

Abstract:

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Presented Published

Pfaller, S.L., and Covert, T.C. Micobacterium paratuberculosis and nontuberculous mycobacterial in potable water. Presented at: U.S. EPA's Research on Micoorganisms in Drinking Water Workshop, Cincinnati, OH, August 5-7, 2003. 8/5/2003

Contact: Stacy L. Pfaller

Abstract: Nontuberculous mycobacteria (NTM) include Mycobacterium species that are not members of the Mycobacterium tuberculosis Complex. Members of the NTM group are important causes of disease in birds and mammals. Mycobacterium avium, Mycobacterium intracellulare and Mycobacterium paratuberculosis are NTM and members of the Mycobacterium avium Complex (MAC). These organisms are found in a variety of environments including soil and water and are included on the Contaminant Candidate List (CCL). Earlier exploratory occurrence studies suggest that NTM have widespread occurrence in potable water throughout the U.S. M. paratuberculosis is the causative agent for Johne's disease in cattle. In addition to well - documented evidence of M. paratuberculosis as the causative agent of Johne's disease in cattle, there has been evidence linking M. paratuberculosis with Crohn's disease, a chronic inflammatory disease in of the intestinal tract in humans. Transmission of M. paratuberculosis via water contaminated with cattle feces may be one route of infection. Current NTM research focuses on three areas: (1) Development of an improved cultural method for isolation of NTM in drinking water. (2) Development of a rapid PCR multiplex method for detection of MAC organisms in drinking water and (3) development of a molecular method for detection of M. paratuberculosis in water.Improved Cultural MethodGoals/Objectives: Current methods for isolating NTM from environmental samples require harsh decontamination techniques to reduce the levels of background organisms often leading to loss of 50 -70% of the target NTM. The goal of this research is to develop improved selective method(s) which do not use classical decontamination procedures.Approach: The use of antibiotics, dyes, detergents and other growth inhibitors are being examined for their ability to reduce background organisms and permit growth of NTM. A membrane filter method approach has been selected. Screening studies with spiked drinking water samples comparing candidate methods to classical decontamination techniques have been initiated. Candidate methods which permit better recovery of NTM and better reduction of background organisms will be tested with additional recovery studies and analyses of drinking water samples. Preliminary Findings: Various antibiotics, dyes, detergents have been examined using a membrane filter cultural method approach. Thus far an oxidizer has shown promise for better recovery (80%) and reduction of background organisms than the standard accepted cultural method. Significance: A improved cultural method would lead to better estimates of the occurrence of NTM, better estimates of the numbers of NTM in positive samples, and the possibility of recovering NTM unusually sensitive to decontaminating agents. Next Steps: Future research will entail additional NTM recovery studies followed by comparison studies with the standard cultural approach and the improved method with distribution samples. PCR Multiplex MethodGoals/Objectives: Current methods for detection of MAC organisms in drinking water typically take 3 - 8 weeks for completion of analyses with additional time for identification of the organisms. The goal of this research is to develop a rapid PCR multiplex method for detection of M. avium and M. intracellulare.Approach: Drinking water samples (500 ml) are membrane filtered and the filters placed in modified 7H9 broth for seven day enrichment. After enrichment the cells are centrifuged and lysed to harvest the genomic DNA. The DNA is amplified (PCR) using primers specific for M. avium and M. intracellulare and all Mycobacteria. The PCR product is visualized by gel electrophoresis. Preliminary Findings: Sixty samples (reservoir and drinking water) have been analyzed by the standard culture method and the multiplex PCR method. Nine samples were positive by both methods, seven were positive only by multiplex PCR and three were positive only by the cultural method. Significance: The use of multiplex PCR significantly decreases the time for analyses for these organisms, and is able to detect MAC organisms not detected by the culture method. Next Steps: Completion of detection limit studies and additional comparison studies with the standard culture technique using drinking water samples.Method for detection of M. paratuberculosisGoals/Objectives: A new project in our lab involves the development of a molecular detection and quantification method for M. paratuberculosis (MAP) in water. The method will be an important step in determining the significance of exposure to MAP in contaminated water, and may help to establish the link between contaminated water and Crohn's disease.Approach: Current methods of detection, which include culture-based methods, are inadequate. A sixteen to twenty week incubation time is required to grow the organism, during which other microorganisms overgrow the medium. Harsh decontamination procedures used to reduce background organisms also kill a portion of MAP. This work proposes to develop a rapid molecular method to detect and quantify MAP in environmental samples by targeting a genetic molecule specific to MAP. One potential target is the MAP-specific insertion sequence IS900. The element is found only in MAP, and is present in fourteen to eighteen copies per cell. Other possible targets include seven recently discovered MAP-specific gene segments. A quantitative PCR-based method would significantly reduce detection times from approximately sixteen weeks to a few hours.

Pfaller, S.L., and Covert, T.C. Genetic fingerprinting of mycobacterium avium complex (MAC) organisms isolated from hospital patients and the environment. Presented at: American Society for Microbiology, Washington, DC, May 18-22, 2003. 5/18/2003

Contact: Stacy L. Pfaller

Abstract: A particularly pathogenic group of mycobacteria belong to the Mycobacterium avium complex (MAC), which includes M. avium and M. intracellulare. MAC organisms cause disease in children, the elderly, and immuno-compromised individuals. A critical step in preventing MAC infections is identifying the source of infection and preventing exposure to that source. The standard method for tracking MAC infection outbreaks is long restriction fragment length polymorphism analysis using pulsed-field gel electrophoresis (PFGE). The method is labor-intensive and may lack sensitivity. The purpose of this study was to develop a rapid, reproducible, and efficient method for fingerprinting mycobacteria at the strain level. Mycobacteria isolates were obtained from AIDS and non-AIDS patients, as well as drinking water and food sources. The strains were identified as M. avium, M. intracellulare, or "MX" using AccuProbe (GenProbe). One hundred and sixty-six isolates were typed using Amplified Fragment Length Polymorphism (AFLP) analysis. Phylogenetic analysis using maximum parsimony was utilized to determine the genetic relatedness of the isolates. AFLP was able to distinguish between MAC species and differentiate between strains within each species. Furthermore, the method was rapid and highly reproducible. None of the isolates were genetically identical. Several "MX" strains clustered with M. intracellulare, and their identities were confirmed with 16S sequence analysis. For both species of Mycobacterium, most drinking water isolates clustered more closely with each other than with patient or food isolates, suggesting that the harsh decontamination procedures used to isolate mycobacteria from the environment select for a subset of the organisms present. Patient isolates were more genetically diverse.

Pfaller, S.L., and Covert, T.C. Genetic fingerprinting of mycobacterium avium complex (MAC) organisms isolated from hospital patients and the environment. Presented at: American Society for Microbiology, Washington, DC, May 18-22, 2003. 5/18/2003

Contact: Stacy L. Pfaller

Abstract:

Donohue, M.J. Proteomic analysis of allergens from metarhizium anisopliea. Presented at: NHEERL Presentation on Proteomics, Research Triangle Park, NC, July 22, 2003. 7/22/2003

Contact: Maura Donohue

Abstract: The goal of this project is the identification and characterization of allergens from the fungus M. Anisopliae, using mass spectrometry (MS). The US EPA, under the "Children at Risk" program, is currently addressing the problem of indoor fungal bioaerosol contamination. One of the research objectives is to develop a basic understanding of IgE inducing proteins from fungi, using advanced proteomic. The Fungus M. Anisopliae has been used as a bio-pesticide for insect control sijnce the 1800's . Recent studies have shown that exposure to this micro-organism can cause an immediate hyper-immunosensitivity or Type I allergenic rsponse. Matrix assisted laser desorption ionization time-of=flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization mass spectrometry (ESI-MS) were used to analyze the fungal peptides and proteins isolated by 2-D gel electrophoresis and immuno-blot analysis. The MS data on molecular weight, peptide profiles, and amino acid sequence or domain homology to known allergens. This information could lead to the identification of the M. Anisopliae proteins that induce IgE responses, eventually paving the way to prevention or treatment of allergies.

JOURNAL

Melnyk, L.J., Morgan, J.N., Fernando, R., Akinbo, O., and Pellizzari, E.D. Determination of metals in composite diet samples by ICP-MS. Journal of AOAC International 86 (2):439-447 (2003). EPA/600/J-03/296. 3/1/2003

Contact: Lisa J. Melnyk

Abstract: In order to assess an individual's total exposure to contaminants in the environment, it is essential that the contribution of dietary exposure be quantified. As a result, USEPA's National Exposure Research Laboratory has initiated a program to develop methods to measure chemical pollutants in dietary samples collected from individuals. Previous efforts have utilized inductively coupled plasma (ICP)-atomic emission spectrometry (AES) and graphite furnace atomic absorption spectroscopy (GFAAS) techniques for determination of metals in composite diets. However, there is often a trade-off between sensitivity and sample throughput with these techniques. ICP-mass spectrometry (MS) offers sensitvity comparable to or better than GFAAS while retaining sample throughput comparable to ICP-AES. This study evaluated the applicability of ICP-MS techniques for the determination of metals in composite diets. An ICP-MS method for the determination of aluminum, arsenic, barium, cadmium, chromium, copper, lead, manganese, nickel, vanadium, and zinc is presented. The procedure utilizes atmospheric pressure microwave digestion to solubilize analytes in homogenized composite diet samples followed by ICP-MS analysis. Recovery of certified elements from standard reference materials (SRMs) ranged from 92-119% with relative standard deviation (RSD) ranging from 0.4-1.9%. Recovery of elements from fortified composite diet samples ranged from 75-129% with RSDs ranging from 1-11.3%. LODs ranged from 1 - 1700 ng/g, with high values due to significant amounts of certain elements naturally present in composite diets. Results of this study demonstrate that low resolution ICP-MS provides precise and accurate measurements of the elements tested in composite diet samples.

Hayes, S.L., Rice, E.W., Ware, M.W., and Schaefer, III, F.W. Low pressure ultraveiolet studies for inactivation of Giardia muris cysts. Journal of Applied Microbiology 94 (1):54-59 (2003). EPA/600/J-03/077. 1/1/2003

Contact: Frank W. Schaefer

Abstract: Cysts of Giardia muris were inactivated using a low pressure ultravolet (UV) light source. Cyst viability was detemined by both in vitro excystation and animal infectivity. Cyst doeses were counted using a flow cytometer for the animal infectivity experiments. Using in vitro excystation as the viability indicator, fluences as high as approximately 200 mJ/cm(2) did not prevent some cysts from excysting, thus verifying earlier work. Using animal infectivity, UV fluences of 1.4, 1.9 and 2.3 mJ/cm(2) yielded log(10) reductions ranging from 0.3 to greater than or equal to 4.4. Results indicate in vitro excystation is not a reliable indicator of G. muris cyst viability after UV disinfection.

Jan 1, 2003 - Dec 31, 2003

Presented Published

Brinkman, N., Haugland, R.A., Wymer, L.J., Muruleedhara, B., Whitman, R.L., and Vesper, S.J. Evaluation of a rapid, quantitative real-time PCR method for enumeration of pathogenic Candida cells in water. Applied and Environmental Microbiology 69 (3):1775-1782 (2003). EPA/600/J-03/300. 3/1/2003

Contact: Richard A. Haugland

Abstract: Quantitative Real-Time PCR (QRT-PCR) technology, incorporating fluorigenic 5' nuclease (TaqMan?) chemistry, was developed for the specific detection and quantification of six pathogenic species of Candida (C. albicans, C. tropicalis, C. krusei, C. parapsilosis, C. glabrata and C. lusitaneae) in water (Brinkman et al, 2001). Known numbers of target cells were added to distilled and tap water samples, filtered and the DNA extracted. The assays sensitivities were between one and three cells with a two-fold accuracy around the mean at a 95% confidence interval. In similar tests with surface water samples, the presence of QRT-PCR inhibitory compounds necessitated further purification and/or dilution of the DNA extracts with resultant reductions in sensitivity but not quantitative accuracy. Analyses of a series of freshwater samples collected from a recreational beach showed positive correlations between the QRT-PCR results and colony forming counts of the corresponding target species. Positive correlations were also seen between the cell quantities of the target Candida species detected in these analyses and colony counts of Enterococcus. With a sample processing time of less than three hours, this method shows great promise as a tool for rapidly assessing potential exposures to waterborne pathogenic Candida species from drinking or recreational water and which may be an alternative indicator of fecal pollution._____Yeasts are a significant component of the micro biota of most natural water systems and can also occur in drinking water distribution systems as a result of their ability to survive treatment practices and become incorporated into biofilms (Woollett and Hedrick, 1970). The majority of these organisms have no known human health effect, however, a small number of species, primarily within the anamorphic genus Candida, are important opportunistic pathogens (Hurley, de Louvi and). The importance of pathogenic Candida as agents of nocosomial infections has led to the development of a number of modern molecular diagnostic methods to facilitate their detection and identification in clinical samples (Reiss et al). Methods based on the polymerase chain reaction (PCR) and DNA hybridization probes have received particular attention (Mannarelli ??; Martin??; Widjojoatmodjo et al 1999). The more recent advent of fluorescent probe-based, real-time PCR (RT-PCR) technology (Heid et al 1996) has led to the development of homogeneous methods for detecting these organisms that require relatively short periods of time to perform (Guiver, Levi Oppenhaimer). Quantitative Real-Time PCR (QRT-PCR) has been demonstrated to be useful for quantitative analysis of microorganisms (Roe et al 2001), but, to our knowledge, this approach has not been used in the analysis of yeasts in water. Analyses for pathogenic yeasts in drinking or recreational water systems have the potential to expedite the identification of possible health hazards resulting either directly from the presence of these organisms or as indicators of other waterborne pathogens. The first objective of this study was to develop QRT-PCR technology, incorporating fluorigenic 5' nuclease (TaqMan) chemistry, for specifically detecting and quantifying six common pathogenic species of Candida including: C. albicans, C. tropicalis, C. krusei, C. parapsilosis, C. glabrata and C. lusitaneae. The second objective was to evaluate a simple and rapid method, using QRT-PCR, for the detection and enumeration of these organisms in different types of water samples. Finally, the technology was tested by comparing this methodology with conventional plating and culturing methods in the analysis of a series of freshwater samples collected from a recreational beach on Lake Michigan.

Anderson, A.D., Heryford, A.G., Sarisky, J.P., Higgins, C., Monroe, S.S., Beard, S.R., Newport, C., Cashdollar, J.L., Fout, G.S., Robbins, D.E, Seys, S.A., Musgrave, K.J., Bartkus, J., Vinje, J., Bresee, J.S., Mainzer, H.M., and Glass, R. A waterborne outbreak of Norwalk-like virus among snowmobilers - Wyoming, 2001. Journal of Infectious Diseases 187 (2):303-306 (2003). EPA/600/J-03/113. 1/15/2003

Contact: G. shay Fout

Abstract: In February 2001, episodes of acute gastroenteritis were reported to the Wyoming Department of Health from persons who had recently vacationed at a snowmobile lodge in Wyoming. A retrospective cohort study found a significant association between water consumption and illness, and testing identified Norwalk-like virus (NLV) in 8 of 13 stool samples and 1 well. Nucleotide sequences from the positive well-water specimen and 6 of the positive stool samples were identical. This multistrain NLV outbreak investigation illustrates the importance of NLV as a cause of waterborne illness and should encourage monitoring for

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Fout, G.S., Martinson, B.C., Moyer, M.W.N., Dahling, D.R., and Wymer, L.J. A multiplex reverse transciption-PCR method for detection of human enteric viruses in groundwater. Applied and Environmental Microbiology 69 (6):3158-3164 (2003). EPA/600/J-03/319. 6/1/2003

Contact: G. shay Fout

Abstract: Untreated groundwater is responsible for about half of the waterborne disease outbreaks in the United States. Human enteric viruses are thought to be leading etiological agents of many of these outbreaks, but there is relatively little information on the types and levels of viruses found in groundwater. To address this problem, monthly samples from 29 groundwater sites were analyzed for one year for enteroviruses, hepatitis A virus (HAV), Norwalk virus, reovirouses and rotaviruses by multiplex reverse transcription-PCR (RT-PCR). A procedure was developed to remove environmental inhibitors of RT-PCR from groundwater samples. The procedure allowed an average of 71 liters of the original groundwater to be asssayed per RT-PCR reaction with an average virus recovery rate of 74%, based upon seeded samples. Human enteric viruses were detected in 16% of the groundwater samples analyzed with reoviruses being the most frequently detected virus group.

Lindquist, H.D.A., Varma, M., Schaefer, III, F.W., Ware, M.W., and Hester, J.D. Detection of cyclospora cayetanesis using a quantitative real-time PCR assay. Journal of Microbiological Methods 53 (1):27-36 (2003). EPA/600/J-03/343. 4/1/2003

Contact: H. d. alan Lindquist

Abstract: Cyclosporal cayetanensis, a coccidian parasite of humans, has been recognized worldwide as an emerging pathogen in both immunocompromised (Ortega et al.1993) and immunocompetent individuals (Berlin et al.1994). Presently, humans apear to be the primary host for this parasite (Eberhard et al.2000). Clinical manifestations associated with C. cayetanensis can include prolonged diarrhea, nausea, abdominal cramps, anorexia, weight loss, and other symptoms of gastronenteritis. The transmission form of C. cayetanensis is an environmentally resistant, 8-10 um spherical oocyst that contains two ovoid sporocysts each of which contains two sporoxoites. Cyclospora cayetanensis oocysts shed in the feces of an infected host are not infectious until they become sporulated. Depending on a variety of environmental factors such as temperature and humidity, sporulation occurs after approximately two weeks outside the host.A striking feature of this parasite is its seasonality. In some areas, during the rainy season, infection rates are high, decreasing to undetectable levels during the dry season. While the mode of transmission has not been completely elucidated, most cases of cyclosporiasis in the United States have been associated with consumption of fruits and vegatables that may have become contaminated after contact with tainted water (Berline et al.1994; Herwaldt and Ackers 1997). Since the epidemiology of this disease is still in doubt, it would be valuable to have sensitive and specific tools to detect and quantify the presence of this organism in the environment. These tools might also lead to the development of more sensitive and specific clinical diagnostic methods.

Van Emon, J.M., Reed, A.W., Yike, I., and Vesper, S.J. ELISA Measurement of Stachylysin (TM) in serum to quantify human exposures to the indoor mold Stachybotrys chartarum. Journal of Occupational and Environmental Medicine 45 (6):582-591 (2003). EPA/600/J-03/320. 6/13/2003

Contact: Stephen J. Vesper

Abstract: Antibodies were produced against the hemolytic agent stachylysin obtained from the mold Stachybotryis chartarum. These antibodies were used to develop two enzyme-linked immunosorbent assay (ELISA) methods for the analysis of stachylysin in human and rat sera and environmental samples. ELISA (I) exhibited a linear range of 10 - 300 ng/mL with a limit of detection of 20 ppb for sera and environmental samples. ELISA (II) was used for the detection of low level samples and had a linear range of 1-30 ng/mL. ELISA (I) was used to measure stachylysin in the conidia of indoor fungi. Stachylysin appears to be specific to S. chartarum. Three week old rat pups received nasal instillations of S. chartarum conidia. Stachylysin was detected in their serum; however, control rat serum had no measurable stachylysin. The serum of five adults exposed to S. chartarum in water damaged, moldy environments was pooled and analyzed using ELISA (II). Results were compared to pooled serum from five adults with no known exposure. Serum stachylysin was measured at 371 ng/mL from the exposed group but was not detected in the serum from the control group. The concentrations of stachylysin from wallboard, mycelial and dust samples were also quantified by ELISA. The measurement of stachylysin may be a useful indicator in assessing human exposure to S. chartarum and to determine the presence of the mold in environmental samples.

Vesper, S.J., Gregory, L., Dearborn, D.G., and Yike, I. Immunocytochemical localization of stachylysin in stachybotrys chartarum spores and spore-impacted mouse and rat lung tissues. Mycopathologia 156 (2):109-117 (2003). 3/1/2003

Contact: Stephen J. Vesper

Abstract: Stachylysin is a proteinaceous hemolytic agent that is producted by S. chartarum. Stachylysin was found, using immunohistochemistical and immunocytochemical methods, to be localized in S. chartarum spores/mycelia primarily in the inner wall suggesting that it is constitutively produced. Spores instilled in mouse or rat lung tissues resulted in granuloma formations which showed the highest stachylysin concentration in the inner wall of the spore, with less at distance indicating that it has diffused out from the spore. The in vitro high stachylysin producing strain (58-06) was also highest in vivo, based on immunohistochemistical staining. More stachylysin was observed in the lung tissue at 72 h than at 24 h indicating that production /release is a relatively slow process. The localization of stachylysin in macrophage phagolysosomes suggests that these cells may be involved with hemolysin inactivation. This would be consistent with what is know about asp-hemolysin produced by Aspergillus fumigatus.

Yike, I., Vesper, S.J., Tomashefski, Jr., J.F., and Dearborn, D.G. Germination, viability and clearance of stachybotrys chartarum in the lungs of infant rats. Mycopathologia 156 (2):65-67 (2003). 3/1/2003

Contact: Stephen J. Vesper

Abstract: The fungus Stachybotrys chartarum has been associated with many adverse health effects including the condition known as idiopathic pulmonary hemorrhage in infants. In order to gain some insight into possible mechanisms, viable conidia of S. chartarum were instilled into the lungs of 4 and 14 day-old rat pups. Germination was observed in the lungs by 24 h in the 4 day old but not until 72 h (and then only rarely) in the 14 day-old pups. Even two weeks after instillation of the conidia, the fungus could still be detected in the lung homogenates, both by dilution plating and quantitative PCR analysis.In the 4 day-old pups, pulmonary inflammation with hemorrhagic exudates was observed and resulted in about 15% mortality rate compared to 0% for the controls instilled with phosphate buffered saline. In the 4 day-old pups, after 3 days, acute neutrophilic inflammation and intense interstitial pneumonia with poorly formed granulomas associated with fungal hyphae and conidia were obvious. The surviving experimental pups showed significantly slower weight gain for seven days. However, 14 day-old rat pups showed neither the lethal effects of exposures to instilled S. chartarum conidia nor the slower weight gain. Rat pup age may determine the response to exposures to S. chartarum conidia with the youngest pups being less able to clear the conidia resulting in more severe health effects.

SYMPOS/CONF

Lye, D.J. Application of USEPA's drinking water regulations towards rainwater catchment systems. Presented at: ARCSA Conference, Austin, TX, August 21-23, 2003. 8/21/2003

Contact: Dennis J. Lye

Abstract: Rainwater harvesting is receiving increased attention worldwide as an alternative source of drinking water. Although federal agencies such as the USEPA acknowledge the existence of rainwater collection systems, the monitoring of this water source is still typically carried out by individual state or regional health agencies. States such as Texas, Ohio, and Hawaii are developing guidelines for the use and maintenance of these types of systems. It is most likely that this water source will eventually be regulated like other public drinking water sources according to the U.S. Safe Drinking Water Act of 1986. Whenever governmental agencies become involved in regulating systems, there are a number of challenges in complying with their sometimes complex regulations. Under present guidelines, collected rainwater will be characterized as a type of surface water. Existing EPA regulations for surface water sources will be discussed along with steps users can take to understand what will be needed to meet this type of regulatory activity. This is an abstract of a proposed presentation and does not necessarily reflect the United States Environmental Protection Agency (EPA) policy. The actual presentation has not been peer reviewed by EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.